ESTONIAN UNIVERSITY OF LIFE SCIENCES
Institute of Veterinary Medicine and Animal Sciences

Tuula Talvikki Sihvonen

IMPLEMENTATION OF BIOSECURITY MEASURES AND ASSOCIATION WITH HERD-LEVEL PREVALENCE OF SELECTED ENDEMIC INFECTIOUS DISEASES IN ESTONIAN DAIRY CATTLE HERDS

BIOTURVALISUSE MEETMETE RAKENDAMINE JA SEOS VALITUD ENDEEMILISTE INFEKTSIOONIHAIGUSTE LEVIMUSEGA EESTI PIIMA VEISEKARJADES

Final Thesis
Curriculum in Veterinary Medicine

Supervisor: Associate professor Kerli Mõtus, *DVM, PhD*

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Eesti Maaülikool
Estonian University of Life Sciences

Estonian University of Life Sciences
Kreutzwaldi 1, 51014 Tartu, Estonia

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Author: Tuula Talvikki Sihvonen

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Bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), *Mycoplasma bovis* (*M. bovis*), *Salmonella* Dublin (*S.* Dublin) and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) impairs animal health, productivity and cause economic loss. Biosecurity measures prevent disease introduction to herds. The aims of this thesis were to investigate the implementation of biosecurity measures, reveal the herd prevalence of BHV-1, BVDV, BRSV, *M. bovis, S.* Dublin and MAP infections and to analyze the association between herd biosecurity measures and pathogens in 120 large-scale Estonian dairy herds. From each herd, ten heifers' blood samples and bulk tank milk samples were analyzed for disease antibodies using ELISA. The herd seroprevalence for BHV-1 was 56.7 % (95 % CI 47.3; 65.7), *M. bovis* 48.3 % (95 % CI 39.1; 57.6), *S.* Dublin 24.2 % (95 % CI 16.8; 32.8) and MAP 2.5 % (95 % CI 0.5; 7.1) for all study herds and for BVDV 27.0 % (95 % CI 19.0; 36.3) and BRSV 94.7 % (95 % CI 88.1; 98.3) excluding the vaccinated herds. Visitors using protective clothing were associated with the lower probability of a herd to be infected with BHV-1, BVDV and *S.* Dublin. Hand disinfection and support service disinfecting their equipment were associated with lower odds of a herd to be positive for *M. bovis*. This study revealed that large-scale Estonian dairy cattle herds are endemically infected with important cattle pathogens and implementation of biosecurity measures could reduce the herd risk for different important cattle pathogens.

Keywords: biosecurity, infectious diseases, dairy herds, protective clothing, disinfection

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Veiste herpesviirus 1 (VHV-1), veiste viirusdiarröa viirus (VVDV), veiste respiratoor-süntsütsiaalviirus (VRSV), *Mycoplasma bovis* (*M. bovis*), *Salmonella* Dublin (*S.* Dublin) ja *Mycobacterium avium* subsp. *paratuberculosis* (MAP) kahjustavad loomade tervist ja tootlikkust ning põhjustavad põllumajandustootjatele majanduslikku kahju. Haiguse leviku tõkestamiseks karjade vahel rakendatakse bioturvalisuse meetmeid. Lõputöö eesmärkideks oli uurida bioturvalisuse meetmete rakendamist, VHV-1, VVDV, VRSV, *M. bovis*, *S.* Dublin ja MAP karjalevimust ning seost bioturvalisuse meetmete rakendamise ja uuritud patogeenide vahel 120 suures Eesti piimaveisekarjas. Igast karjast uuriti kümne mullika vereproove ja tankipiimaproove nimetatud haiguste antikehade suhtes kasutades ELISA meetodit. Karjas rakendatavate bioturvalisuse praktikate ja vaktsineerimisandmete registreerimiseks kasutati küsimustikku. VHV-1 karjalevimus oli 56.7 % (95 % CI 47.3; 65.7), *M. bovis* 48.3 % (95 % CI 39.1; 57.6), *S.* Dublin 24.2 % (95 % CI 16.8; 32.8) ja MAP 2.5 % (95 % CI 0.5; 7.1). Jättes kõrvale vaktsineerivad karjad, oli VVDV karjalevimus 27.0 % (95 % CI 19.0; 36.3) ja VRSV 94.7 % (95 % CI 88.1; 98.3). Külastajate kaitseriietuse ja –jalanõude kandmine seostus karja väiksema tõenäosusega olla nakatunud VHV-1, VVDV ja *S.* Dublin nakkusega. Käte desinfitseerimisvahendite olemasolu farmi sissepääsu juures ja teenusepakkujate varustuse desinfitseerimine seostus karja väiksema *M. bovis* nakkusriskiga. Käesolev uuring näitab, et Eesti suured piimaveisekarjad on endeemiliselt nakatunud mitmete oluliste veiste patogeenidega ja bioohutusmeetmete rakendamine võib vähendada mitmete oluliste infektsioonhaiguste esinemise riski karjas.

Märksõnad: bioturvalisus, nakkushaigused, piimakajad, kaitseriietus, desinfitseerimine

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LIST OF ABBREVIATIONS

AI-technician	Artificial insemination technician
BHV-1	Bovine herpesvirus 1
BSM	Biosecurity measure
BRSV	Bovine respiratory syncytial virus
BTM	Bulk tank milk
BVDV	Bovine viral diarrhea virus
DIVA	Differentiating infected from vaccinated animals
ELISA	Enzyme-linked immunosorbent assay
gB	Glycoprotein-B
gE	Glycoprotein-E
IBR	Infectious bovine rhinotracheitis
IPB	Infectious pustular balanoposthitis
IPV	Infectious pustular vulvovaginitis
M. bovis	*Mycoplasma bovis*
MAP	*Mycobacterium avium* subsp. *paratuberculosis*
PI	Persistent infection
S. Dublin	*Salmonella* Dublin

INTRODUCTION

BHV-1, BVDV, BRSV, *M. bovis*, *S.* Dublin and MAP cause endemic infections in dairy cattle worldwide. Several European countries have managed to eradicate some of the infectious diseases with test and cull-strategy and vaccination programs (MacLachlan and Dubovi, 2017). Infectious diseases have important economic impact for cattle production. Severe direct and indirect losses are caused by reduced growth and/or production level, impaired fertility, or increased susceptibility to other diseases (Damiaans *et al*., 2020). *S.* Dublin is a zoonosis, so the pathogen is a potential risk for public health (Holschbach and Peek, 2018; Matthews *et al.*, 2015).

In veterinary medicine the focus on treating an individual animal is moved towards the maintenance of animal's health and welfare on a herd-level. Implementing biosecurity measures on a farm have an important role in case of infectious diseases prevention on a farm (Sarrazin *et al.*, 2014). Biosecurity is a wide concept including all the measures from the level of national government to an individual farm operator for preventing disease agents from entering to the herd and spreading within the herd (Damiaans *et al*., 2020).

Purchasing of cattle and participation in animal shows are remarkable direct ways to introduce a pathogen to herd (Benavides *et al*., 2020), but the risks can be decreased by purchasing animals only from farms with a known disease history, through quarantine and perform disease testing prior to animal movement (Brennan and Christley, 2012). The number of people visiting in a farm increases with increasing herd size, so in larger herds pathogens have a higher chance to enter the farm by indirect contacts compared to smaller herds (Nöremark *et al*., 2013). Providing protective clothes and footwear for visitors combined with washing and disinfecting procedures will lower the risk of human-mediated disease transmission between herds (Oma *et al*., 2018).

Biocheck-system is a risk-based scoring system to assess relative importance of farm's biosecurity management and benchmark farms by comparing the own farm results to the national or international averages. In future Biocheck is probably applied more also on cattle farms to motivate farmers in better disease prevention. (Damiaans *et al*., 2020).

Previous broader studies concerning the prevalence of several economically important cattle pathogens and their associations with biosecurity measures in Estonian dairy cattle herds has

not established. The present study gives an overview of the selected cattle pathogens and their current prevalence estimates in large-scale Estonian dairy cattle herds. Also, the study raises of biosecurity awareness of farmers, employees, and other persons contacting with cattle.

The aim of this study was to analyze the implemented biosecurity measures and their associations with herd BHV-1, BVDV, BRSV, *M. bovis*, *S.* Dublin and MAP infection status on large-scale commercial Estonian dairy cattle herds.

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1. LITERATURE ANALYSIS

1.1. Bovine herpesvirus 1

1.1.1. Etiology and pathogenesis

Bovine herpesvirus type 1 (BHV-1) causes diseases called infectious bovine rhinotracheitis (IBR), which is also known as red nose disease, infectious pustular vulvovaginitis (IPV) in cows and infectious pustular balanoposthitis (IPB) in bulls (Divers and Peek, 2008). BHV-1 can be subdivided into BHV-1.1, BHV-1.2 and BHV-1.3, which are antigenically similar pathogens. BHV-1.1 is usually associated with respiratory tract disease and in case of subtype BHV-1.2 genital tract lesions are common. BHV-1.3 was reported to cause encephalitis usually in calves and now the subtype is referred as BHV-5 with manifestation of neurological disorders (Muylkens *et al*., 2007; Biswas *et al*., 2013). Based on localization of BHV-1 clinical signs, it can be divided into two forms: respiratory and reproductive. Respiratory form of the infection is more common, and cattle are the primary reservoir of the virus (Divers and Peek, 2008).

Periodic or continuous shedding of the virus is typical for all viruses belonging to the family Herpesviridae. Infection is said to be persistent, so the virus is never eliminated from the host. Latent infection is a specific type of persistent infection and means that the virus exists but is not exhibited. Main site for life-long herpesvirus latency is trigeminal ganglia or pharyngeal tonsils in respiratory form and sciatic ganglia in reproductive form. Reactivation of the virus is associated with stress, which can be caused by intercurrent infections, transport, cold, overcrowding, or by administration of glucocorticoid drugs (Fulton *et al*., 2013; MacLachlan and Dubovi, 2017).

1.1.2. Clinical signs

Upper respiratory tract disease signs are seen in the respiratory form of the herpesvirus infection. Typical clinical signs of IBR include fever, nasal discharge, conjunctivitis, profuse lacrimation, inappetence and depression. Also, a dried necrotic crusting of the muzzle, white plaques visible in the nasal mucosa, dyspnea, mouth breathing, salivation, a deep bronchial cough and decrease in milk yield are seen in cattle suffering from IBR (MacLachlan and Dubovi, 2017). Clinical signs usually manifest for 7 to 14 days and animals recover from those due to the rise of specific immune response (Divers and Peek, 2008).

Genital form of the herpesvirus infection can be seen as swelling of genital organs, discharge from vulva/penis and small pustules in mucosa of genital organs. BHV-1 infected pregnant cows may abort usually at 4-7 months of gestation. This genital disease is rarely diagnosed at the same time with respiratory disease (MacLachlan and Dubovi, 2017).

1.1.3. Epidemiology

IBR distribution is worldwide (Figure 1), but several countries, i.e., Denmark, Finland, Sweden, Norway, Switzerland, Austria, the Province of Bolzano in Italy (2011/674/EU) and the Federal State of Bavaria in Germany (2011/674/EU) have eradicated the disease. Eradication programs are based on either detection and culling of the seropositive animals or on the repeated vaccination of infected herds with marker vaccines (MacLachlan and Dubovi, 2017; Ackermann and Engels, 2006).

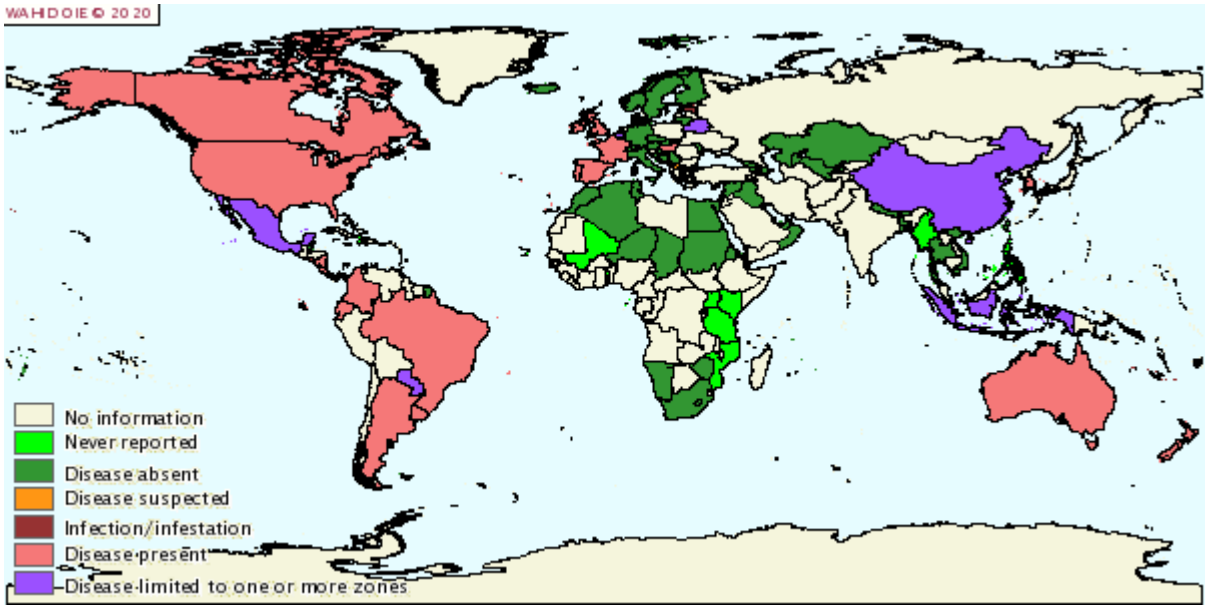

Figure 1. IBR/IPV distribution map in July-December 2019 in domestic bovines (OIE, 2020).

IBR is mainly a herd problem and mostly occurs in animals over six months of age (Fulton *et al*., 2013). Infected animals shed the herpesvirus in nasal, oral and genital secretions. Transmission is usually with direct mucosal contact, but droplet and fomite infections are also common (MacLachlan and Dubovi, 2017). A fomite is referred as object which may be contaminated with infectious organisms and transmit pathogens to susceptible animals (Stevens *et al*., 2016). Cows may get the infection from BHV-1 seropositive bulls during mating or artificial insemination. IBR is found more in feedlots and in intensive dairy farms than in free-range cattle. Moist and cool environmental conditions favor the virus survival (MacLachlan and Dubovi, 2017). Raaperi *et al*. (2010) has shown that herd prevalence of BHV-1 increases

significantly with herd size in Estonian dairy herds. The same study concluded that the mean within-herd prevalence also increased with herd size. Airborne transmission has been demonstrated over short distances which can explain some between-herd transmission across farm boundaries (Studdert, 2010). Morbidity of IBR is high, but mortality low unless there occur significant complications with a secondary infection for example with bovine viral diarrhea virus (MacLachlan and Dubovi, 2017).

1.1.4. Diagnosis

Bulk-tank milk (BTM) testing is considered fast, easily available and inexpensive testing method of BHV-1 at herd-level compared to blood sampling. Testing of BTM is non-invasive, so stress is not caused for the animals and large number of cattle can be tested simultaneously (Reber *et al*., 2012). Serological test is used for definitive diagnosis of IBR or IPV infection. Enzyme-linked immunosorbent assay (ELISA) can detect antibodies in serum or plasma and with lower sensitivity in milk or bulk tank milk samples. The herd is not defined as BHV-1 free based on the negative bulk or pooled milk samples, so individual blood samples are taken in order confirm the diagnosis in diseased animals (Beer and Dastjerdi, 2019). Seroconversion is interpreted from the results of paired serology samples taken on day 1 from acutely infected animals and in day 14 (Peek and Divers, 2018). The virus can also be isolated from the respiratory or genital tract samples or from aborted fetuses. BHV-1 seropositive animals are considered as latently infected (Beer and Dastjerdi, 2019).

1.1.5. Control

Test-and-slaughter strategy is used to control BHV-1 by gradually removing seropositive animals and replacing them with seronegative progeny in herds with low seroprevalence of the virus (Raaperi *et al*., 2014; Ackermann and Engels, 2006). Differentiation of infected from vaccinated animals (DIVA)- strategy is another method to control BHV-1 infection in a herd. Vaccination do not completely prevent the infection but prevent the development of clinical signs and reduce the shedding of the virus after infection. In DIVA-strategy cattle are vaccinated with attenuated or inactivated marker vaccines. Those vaccines are based on deletion of glycoprotein E (gE) or on a subunit of virion for example glycoprotein D. Field virus infected cattle is distinguished from cattle vaccinated with a gE-deleted marker vaccine with use of a gE-antibody-ELISA (Beer and Dastjerdi, 2019).

1.2. Bovine viral diarrhea virus

1.2.1. Etiology and pathogenesis

Bovine viral diarrhea virus (BVDV) is highly infectious and contagious cattle disease. BVDV belongs to the family Flaviviridae and genus Pestivirus. The virus can cause gastrointestinal, respiratory, or reproductive disease. BVDV classification is based on genotypes 1 and 2. Both genotypes have two biotypes, which can be distinguished from each other based on whether they cause cytopathic effect or not. In cell culture the cytopathic BVDV causes vacuolization and death of certain cell lines within days of inoculation. Noncytopathic BVDV is more prevalent biotype in cattle and inoculation results in persistent infection in cells without obvious cytopathology. Persistent infection arises in the individual animal when noncytopathic virus have infected the fetus before 125 days of gestation (Divers and Peek, 2008). Infection at 80-150 days of gestation can result destructive fetal lesions like retinal dysplasia, cerebellar hypoplasia or hydranencephaly and retardation in growth that results in fetal death or low birth weight. Embryonic death and resorption often occur when pregnant cow is infected prior to 40 days of gestation. Calves that survive *in utero* infection when infected up to 125 days of gestation will remain persistently infected carriers for the rest of their lives and do not get effective immune response to the virus. Fetuses affected after 125 days of gestation usually survive and develop neutralizing antibodies and eliminate the virus. Abortion can occur at any stage of gestation. Genotypes of BVDV can result two clinically different syndromes called bovine viral diarrhea or mucosal disease from which the latter is more severe. Mucosal disease evolves to an individual which is superinfected with both noncytopathic and cytopathic viral biotypes that are genetically homologous (MacLachlan and Dubovi, 2017).

1.2.2. Clinical signs

Infecting virus strain and the gestation stage at infection affects the result of the BVDV infection. It can be either embryo/fetal death, teratogenesis, persistent infection (PI), or inapparent infection with an immune response. Calves have colostrum-derived “passive” immunity, which disappear by three to eight months of age so clinical signs of BVDV may show up mostly after the level of maternal antibodies are waned (MacLachlan and Dubovi, 2017). BVDV causes multiple clinical signs which can be biphasic fever, diarrhea, depression, reduced appetite, and decreased milk yield. Focal or multifocal erosions in the oral cavity and digital lesions are the only “lesions” that are seen (Divers and Peek, 2008). According to Amelung *et al*. (2018) there was no difference in average annual milk yield between BVDV-

positive and BVDV-negative herds. Clinical signs of mucosal disease resemble those in BVDV, but signs are more severe. In addition to fever and anorexia, also the signs of profuse watery diarrhea, nasal discharge, severe erosive or ulcerative stomatitis, dehydration, emaciation and even death can occur (MacLachlan and Dubovi, 2017).

1.2.3. Epidemiology

BVDV is distributed worldwide (Figure 2) and some European countries including Denmark, Norway, Sweden, and Finland have eradicated or are almost free from the virus (Wernike *et al*., 2017). BVDV infection is most common in young animals in herd even though cattle of all ages are susceptible (MacLachlan and Dubovi, 2017).

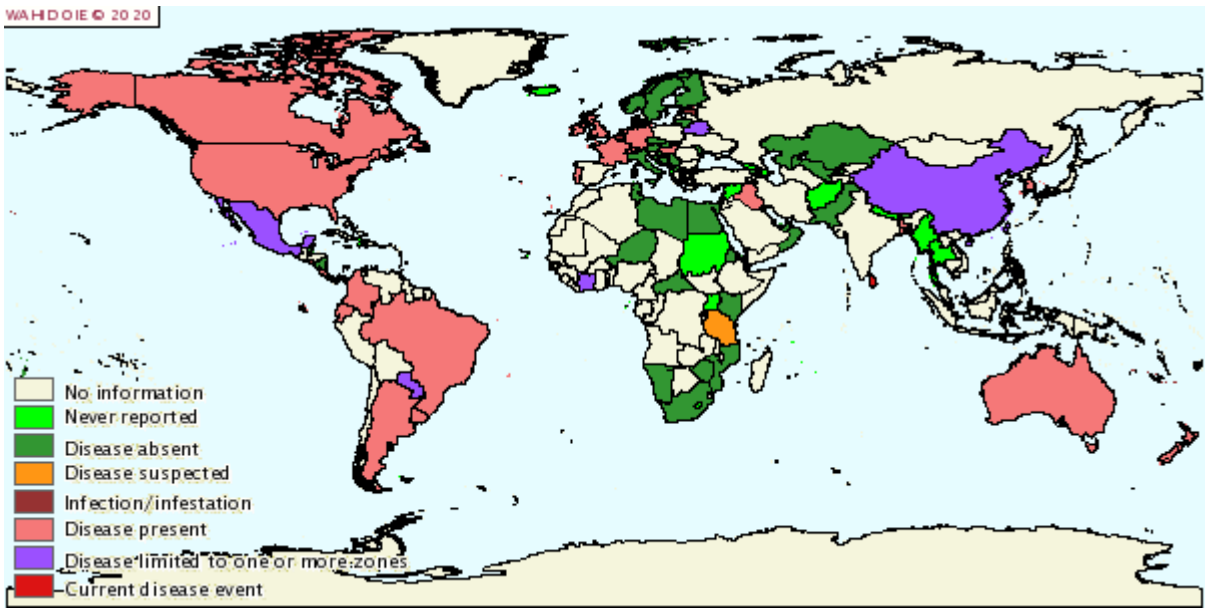

Figure 2. BVDV distribution map in July-December 2019 in domestic bovines (OIE, 2020).

Transmission of the virus can be both vertical and horizontal. Vertical infection can occur during pregnancy and lead to congenital infection of the fetus as the virus can cross the placenta. Horizontal infection can happen by direct-contact, fomite contamination of feed or water and by aerosols at short distance. The virus can be introduced to the herd also by contaminated vaccines and embryo transfer reagents. Disease patterns vary markedly within and between herds, depending on herd immunity and presence or absence of persistently infected (PI) animals. The virus is efficiently transmitted from PI animals rather than acutely affected cattle. PI animals remain seronegative and shed the virus in all body secretions and excretions throughout their lifetime to the susceptible cattle in the herd (MacLachlan and Dubovi, 2017). Larger herds have higher risk for BVDV infection than smaller herds. It was shown that for

each 100 additional animals per farm the odd for infection increased about 50 % (Amelung *et al*. (2018).

1.2.4. Diagnosis

PI animals not always show clinical signs, so infection may stay unnoticed without herd surveillance testing at routine intervals (Newcomer and Givens, 2016). Different diagnostic tests are available for detection of BVDV virus, antigen (Ag) or antibody (Ab) immunological tests. Result of the test will depend on the current or previous BVDV infection status. Animal that has never been exposed to the virus will test negative for virus, Ag and Ab. Animal or late-term fetus that has experienced an acute infection will test Ab positive and Ag or virus negative. PI animal will test Ag or virus positive and Ab negative (Lanyon *et al.*, 2014). In a herd level diagnosis of BVDV antibodies or virus can be detected in BTM. Real-time polymerase chain reaction (RT-PCR) is used to detect virus in BTM and distinguish BVDV-infected animals among lactating cows at individual testing (Houe *et al*., 2006). Non-lactating animals are tested by pooled ear-notch or blood samples for PCR virus detection (Newcomer and Givens, 2016). Antibody detection in BTM is useful for classification of herd BVDV status. BTM antibody testing has high sensitivity and low specificity in herds with PI animals, so it identifies almost all true-positive herds but also detects several false-positive herds.

False-negative results will be obtained in recently infected herds in which only a few animals have seroconverted. Other herd level diagnostic tests are done in individual or pooled serum/plasma samples from youngstock or pooled samples from primiparous cows. Antibody levels in BTM decrease slowly compared to serum antibody tests of youngstock. “Spot testing” meaning to test few animals older than 6-8 months is the key to identify herds that may have received false-positive results in BTM test as it indirectly indicates presence or absence of PI animals in a herd. Animals selected for spot testing need to be representative of the group, so recently introduced animals to the herd or animals that were not part of the herd when they were young, are not involved (Houe *et al*., 2006).

1.2.5. Control

Eradication of BVDV within herds is based on identification and elimination of PI cattle and preventing their further occurrence by quarantine. PI animals are lifelong virus shedders and if husbandry practices remain poor, they can facilitate uninterrupted virus transmission (MacLachlan and Dubovi, 2017). Vaccination is used to prevent both clinical disease after BVDV exposure in cattle and viremia result in transplacental infection and birth of PI offspring.

Vaccination of reproductive-age cattle is considered the most important as it prevents birth of PI calves. Replacement heifers should be fully vaccinated before the onset of the breeding period. Available vaccines are modified-live viral (MLV) and inactivated viral vaccines, which are often combination with other viral and bacterial antigens (Newcomer *et al*., 2017). MLV vaccines stimulate higher levels of antibodies and cell-mediated immunity compared to inactivated vaccines, which are needed to administer multiple doses to achieve protective antibody levels (Woolums *et al*., 2013). Adverse effects of inactivated vaccines for pregnant animal and the developing fetus are lower than MLV vaccines. Multivalent vaccines prevent infection by varied field strains of BVDV and are therefore more current formulations in use compared to monovalent vaccines (Newcomer *et al*., 2017).

1.3. Bovine respiratory syncytial virus

1.3.1. Etiology and pathogenesis

Bovine respiratory syncytial virus (BRSV) is a paramyxovirus that causes rapidly spreading respiratory disease in cattle. State of infection can vary from mild clinical manifestation to severe respiratory disease outbreaks. BRSV can predispose animal to other respiratory tract infections. BRSV damages physical defense mechanisms of the lower airway like mucociliary transport, which makes the underlying tissue vulnerable for infections (MacLachlan and Dubovi, 2017). BRSV is part of the bovine respiratory disease complex (BRD) which includes also BVDV, BHV-1, parainfluenza-3 (PI3), and bacteria *Mannheimia haemolytica, Mycoplasma bovis, Pasteurella multocida* and *Histophilus somni* (Grissett *et al*., 2015).

1.3.2. Clinical signs

Clinical signs of BRSV infection are sudden onset of high fever, depression, anorexia, decreased milk yield, salivation, nasal discharge, and dyspnea ranging from simple increased respiratory rate to open mouth breathing (Divers and Peek, 2008; MacLachlan and Dubovi, 2017). Morbidity due to the infection is high, but mortality is low unless there is also a secondary bacterial infection (Divers and Peek, 2008).

1.3.3. Epidemiology

BRSV occur worldwide in all bovine species as well as in sheep, goats, and other ungulates. During the winter months when cattle are housed the infection is more common. Also, recently weaned young cattle are susceptible especially if they are kept in confined environment.

Transmission of the virus occurs through aerosols or droplets of respiratory tract excretions (MacLachlan and Dubovi, 2017). Important route of virus transmission is by direct animal contact meaning shared pastures or live animal trade. Short distance between herds might be associated with higher risk of direct transmission during pasture time in the summer. Indirect transmission of the virus happens by fomites like clothing or equipment (Toftaker *et al.*, 2016). Disease severity may be reduced if the animal has derived maternal antibodies via colostrum or actively by vaccination or prior infection, but virus can still replicate and be excreted (MacLachlan and Dubovi, 2017).

1.3.4. Diagnosis

BRSV has short life cycle in cattle, which makes it difficult to detect in nasal swabs and post-mortem diagnosis often reveal secondary bacterial infection (Ellis, 2017). BTM serology is cheap and effective method for detecting BRSV in a herd (Toftaker *et al*., 2016). BRSV is diagnosed by detection of virus, viral antigen, or viral RNA in tissues, secretions, or excretions of infected animals. Commercial ELISA tests are used for antibody detection (Brodersen, 2010).

1.3.5. Control

Vaccination is used to control BRSV (MacLachlan and Dubovi, 2017). MLV parenteral vaccines can stimulate disease-sparing, antibody and cell-mediated immune responses. MLV intranasal vaccines administered early in life provide a potentially more effective method to produce protective immunity than parenterally administered vaccines. Duration of the immunity is rather short-lived in intranasal compared to parenteral administration of vaccine. Inactivated BRSV parenteral vaccines are also shown to be effective in cattle (Ellis, 2017).

1.4. *Mycoplasma bovis*

1.4.1. Etiology and pathogenesis

Mycoplasma bovis (*M. bovis*) is the most common subtype of *Mycoplasma* spp. and it infects cattle of all age groups (Divers and Peek, 2008). Mycoplasma is a bacterium lacking a cell wall, which makes it resistant to ß-lactam antibiotics (Haapala *et al*., 2018). Host's immune response is effectively evaded by the high genetic and antigenic variability of the pathogen (Dudek *et al*., 2020). *M. bovis* causes mastitis, respiratory disease, polyarthritis, otitis media/interna, conjunctivitis and abortions. Some infected cows may recover back to production from acute

infection, but others usually develop chronic mastitis or lameness. Characteristic for *M. bovis* is chronic course of the disease and difficulties in successful treatment (Divers and Peek, 2008). Chronic state can cause caseous necrotic lung lesions and make *M. bovis* to spread effectively in the herd (Maunsell and Donovan, 2009; Dudek *et al*., 2020).

1.4.2. Clinical signs

Clinical signs may include acute mastitis, arthritis, respiratory disease, otitis interna, lameness, or reproductive problems (Divers and Peek, 2008). Aebi *et al*. (2015) study showed that the most predominant clinical symptom of *M. bovis* infection was mastitis and the second most common was pneumonia in cows. In case of mastitis, *M. bovis* usually affected two or more quarters of the udder and acute cases were associated with fever. Decreased milk yield was usually seen in acute but not in subclinical mastitis cases (Divers and Peek, 2008).

1.4.3. Epidemiology

Nowadays *M. bovis* infection is common in cattle worldwide (Figure 3). In Finland, *M. bovis* was detected first time in 2012 in calves, which had arrived from several dairy farms to a calf rearing farm. *M. bovis* can be introduced to the naïve herd by artificial insemination with processed semen and the outcome can be cases of clinical mastitis (Haapala *et al*., 2018).

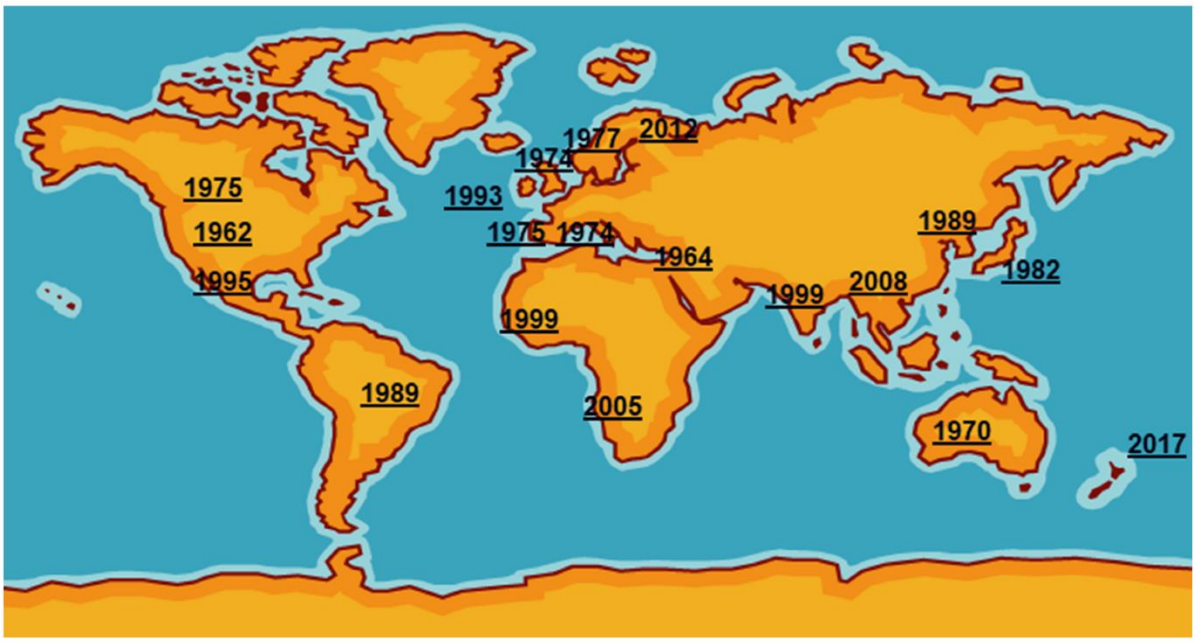

Figure 3. The years of the first detections of *Mycoplasma bovis* around the world (Dudek *et al*., 2020).

Transmission of the infection can be direct by a purchased animal from an infected herd or by mechanical transmission by contaminated workers. Infection may stay unnoticed and spread

via asymptomatic carrier until stress due to e.g., calving or transportation will cause the pathogen secretion (Nicholas *et al*., 2016). According to Haapala *et al*. (2018) the pathogen is often introduced to the herd by healthy carrier animals and there is no supportive evidence of *M. bovis* airborne transmission. Cows at the beginning of lactation are in a greater risk to get mastitis than cows in mid-lactation and the infection spreads like contagious mastitis. Milk contaminated with *M. bovis* can also transmit the infection to calves. Cold and wet environment favor *M. bovis* survival (Divers and Peek, 2008).

1.4.4. Diagnosis

Clinical signs of *M. bovis* are non-specific, so rapid, sensitive and accurate testing of animals is needed for diagnosis. Gold standard method for *M. bovis* diagnosis is culture media, but the method is time-consuming and requires specific conditions. BTM samples, deep nasopharyngeal swabs, lung samples, tracheal aspirate samples and bronchoalveolar lavage can be a good diagnostic material using PCR to confirm the presence of the pathogen. The most accurate diagnosis is got with the combination of culture of viable bacteria and RT-PCR (Dudek *et al*., 2020). In practice, those methods can have low sensitivity due to intermittent shedding patterns and obtaining the best sample materials (Petersen *et* al., 2018). Serum antibody responses can have high level of variation between individual cows, so using ELISA is more useful for herd- or group-level diagnosis of *M. bovis* infection. In BTM samples, antibodies against *M. bovis* were increased only in cows with *M. bovis* mastitis, which indicates that it has diagnostic value for detecting cows having mastitis. In cows having for example arthritis caused by *M. bovis*, antibodies are detected in serum, but the level of antibodies in milk is low (Petersen *et al*., 2018).

1.4.5. Control

The aim of control is identification of infected animals and isolating them from uninfected herd. Teat dipping after milking with 1 % iodine dips and milking machine rinsing will prevent the disease spreading. *M. bovis* affected milk should not be fed to calves. Vaccines against *M. bovis* are available but their effectiveness is controversial (Divers and Peek, 2008). *M. bovis* infection is considered as untreatable and early detection following culling of infected animals is common recommendation for control (Nicholas *et al*., 2016).

1.5. *Salmonella* Dublin

1.5.1. Etiology and pathogenesis

Salmonella Dublin (*S.* Dublin) is one of the serotypes of *Salmonella enterica* subsp. *enterica* and it is adapted to cattle. *S.* Dublin can cause lifelong infection, but cattle might be asymptomatic carriers with intermittent periods of bacteremia and shedding. The most common source of infection to cattle is pathogen-contaminated manure (Holschbach and Peek, 2018).

1.5.2. Clinical signs

S. Dublin causes mastitis in dairy cows and respiratory signs to calves. Mastitis is more likely caused by environmental contamination of the udder rather than septicemic spread to the udder. Calves infected with *S.* Dublin are usually septicemic, and respiratory signs are accompanied by fever. Infected dairy cows can abort at any stage of gestation, because of septicemia, endotoxins or high fever (Divers and Peek, 2008). Host's inflammatory reaction to the infection is mostly seen as diarrhea both in calves and adult cows. Fresh blood can be observed in the feces, because of the inflammation in the colon (Holschbach and Peek, 2018).

1.5.3. Epidemiology

Transmission of *Salmonella* occurs mainly through fecal-oral route, but also colostrum, unpasteurized milk, and respiratory secretions might be the source of infection (Holschbach and Peek, 2018). Nielsen *et al*. (2004) showed that there was higher probability to become carrier of *S.* Dublin if animals became infected as heifers (1 year to 1st calving) and for cows infected around the time of calving (70 days from calving date) than if they became infected during mid or late lactation. Also, the time of the year during infection and the level of exposure influenced whether animals became carriers after infection (Nielsen *et al.*, 2004).

1.5.4. Diagnosis

Fecal culture remains golden standard for *Salmonella* diagnosis. In larger herds it is time-consuming and expensive. Subclinical and persistently infected animals usually shed lower numbers of organisms to feces than clinically ill or acutely infected animals. PCR technique for detecting bacterial genetic material can be done from milk, feces, tracheal or bronchoalveolar lavage fluid. Subsequent serotyping is not always possible with PCR (Holschbach and Peek, 2018). ELISA is used for measuring the level of antibodies directed against O-antigens from *S.* Dublin in serum or milk. A positive BTM sample on bacteriological culture may not represent only the true lactational shedding of the pathogen, but also fecal contamination or both

(Holschbach and Peek, 2018). Sensitivity of individual serum ELISA is higher than fecal bacteriology for detecting *S.* Dublin infected animals (Nielsen, 2013).

1.5.5. Control

Isolation of diseased and carrier animals and environmental hygiene are important to control *Salmonella* infection. Hygiene includes personal hygiene, use of protective clothing and disinfected footwear to all workers and visitors. Attention should be in the high-risk group of animals, which are late gestation and early lactation cows, as they are most susceptible to salmonella infection. Commercial live vaccine administered to newborn calves for *S.* Dublin control is used in the United States. Vaccine should prevent serious health consequences of natural infection and development of carrier status in susceptible animals (Holschbach and Peek, 2018).

1.6. *Mycobacterium avium* subsp. *paratuberculosis*

1.6.1. Etiology and pathogenesis

Paratuberculosis is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) which is extremely stable bacterium in the environment. Clinical paratuberculosis is also known as Johne's disease. It is characterized by progressive granulomatous infection of the intestinal tract shown as chronic diarrhea and emaciation of the infected animal. Incubation period of MAP can be from 2 to 10 years so subclinical stage of the disease is common (Divers and Peek, 2008; Fecteau, 2017).

1.6.2. Clinical signs

Cattle with clinical signs of paratuberculosis have chronic diarrhea, progressive weight loss and hypoproteinemia usually seen as ventral pendent edema in the submandibular area. In early course of the disease, appetite and attitude are normal, but with progressive disease milk production and body condition are decreased. There is no fever and other vital signs are also normal in cattle having paratuberculosis. Cows with moderate to severe paratuberculosis can have abomasal displacement. Age of onset of clinical signs will tell the severity of the infection in herd. If some 2-year-old heifer develop clinical signs, it would suggest a rather heavy dose of MAP at an early age and clinical signs in older cows would suggest low dose of MAP or older age at the time of exposure (Divers and Peek, 2008). According to Fecteau (2017),

subclinically infected animals appear healthy, but they have decreased milk yield and reduced fertility compared with uninfected herd mates.

1.6.3. Epidemiology

Paratuberculosis is distributed throughout the world (Figure 4). Transmission of infection is mainly by fecal-oral route. Calves can easily contract the infection from their dam in utero or via ingestion of contaminated milk or colostrum. Most likely, infection of MAP comes to the herd through purchase of infected animals. As the disease is usually subclinical, the animal may appear serologically negative and fecal culture negative (Fecteau, 2017).

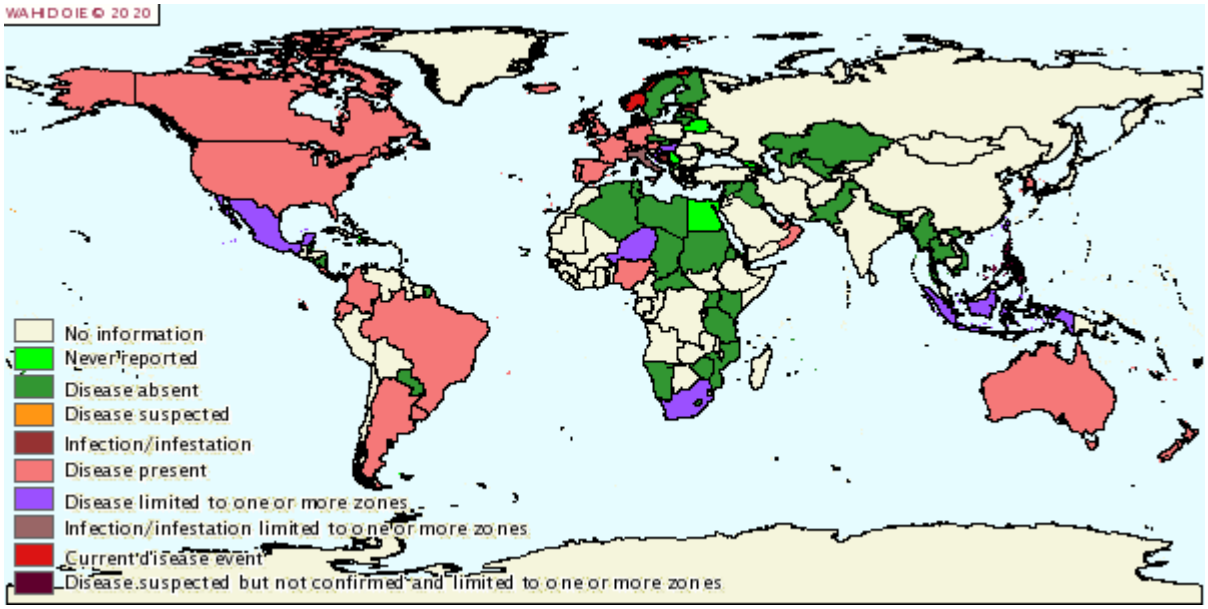

Figure 4. Paratuberculosis distribution map in July-December 2019 in domestic animals (OIE, 2020).

1.6.4. Diagnosis

Diagnosis of paratuberculosis can be divided to immune-based tests to detect antibodies to MAP in serum or milk and detection of MAP organisms or MAP bacterial DNA (Fecteau, 2017). From immune-based tests ELISA is the most sensitive having sensitivity in subclinical animals 15 % and in clinically infected animals around 90 %. Specificity of milk or serum ELISA is around 98 % to 100 % (Collins *et al.*, 2005). With ELISA many samples can be processed at the same time and with relatively low cost compared to culture (Fecteau, 2017). To test BTM with ELISA using the quantitative results like optical density value or sample to positive ratio will recognize the heavy fecal shedders that pose the greatest risk for shedding MAP organism. Organism detection tests and antibody tests are not useful to screen

asymptomatic young cattle under three years old as those animals will test negative even if infected (Divers and Peek, 2018).

1.6.5. Control

Control of paratuberculosis needs long-term management strategies. Negative herds goal is to stay MAP free by closed herd fashion so introduction of new animals should come from disease free herds and participation to auctions or shows should be avoided. Positive herds need to prevent new infections, which most probably occur during neonatal period via the fecal-oral route. Management strategies like separating calving pens for MAP positive and negative cows and separation of calves from cows immediately after birth decrease infection pressure to susceptible cattle. Colostrum only from MAP-negative cows should be fed to calves. Test and cull-strategy may include all positive animals or may be targeting the most infectious cows. MAP-positive cows with some other production disorder like mastitis, lameness or poor fertility should also be culled. Vaccination as controlling paratuberculosis is used mainly in high prevalence herds with good husbandry practices (Fecteau, 2017).

1.7. Biosecurity for controlling cattle pathogens

Biosecurity on a farm can be divided to external and internal biosecurity. External biosecurity includes all measures preventing disease agents from entering to the herd and internal biosecurity is focused on preventing disease agents spreading within the herd. Internal biosecurity can be referred also as biocontainment. Developing biosecurity plans include all the levels from the national government to an individual farm operator. Usually, biosecurity on a farm is assessed with a questionnaire, but the use of computer web-based Biocheck-scoring system to quantify biosecurity has become more common also in cattle production (Dargatz *et al*., 2002; Damiaans *et al*., 2020).

Biocheck-system is a risk-based scoring system to assess relative importance of farm's biosecurity management and benchmark farms by comparing the own farm results to the national or international averages. In future Biocheck is probably applied more also on cattle farms to motivate farmers in better disease prevention. (Damiaans *et al*., 2020).

1.7.1. Biosecurity-related risk factors for cattle infectious diseases

Animal movement between farms increases the risk of disease transmission via direct contact. To reduce this risk farmer can take care of purchasing animals only from farms with a known disease history and through quarantine, disease testing and prophylactic treatment of the animals. Some farmers carry out preventive measures after movement of animals. Those post-movement treatments include vaccination and anthelmintic administration, and some do health checks or disease testing (Brennan and Christley, 2012).

Benavides *et al*. (2020) study showed that farm which had local movement of animals to farms and shared transport with other farms had higher probability for BVDV and BHV-1 introduction than farms which had not implemented that kind of action. Transports where animals are mixed from different farms was quite common and increased the risk of disease introduction in several farms. Gathering of large numbers of cattle and overcrowded barn are stress factors which could result in BHV-1 reactivation and facilitate spread of infectious disease. Farms that attended competitions, there was not a significant probability of disease introduction through animals participating. The probability of disease introduction by competitions reduced when compulsory testing of all attended animals was carried out. The vehicle for animal transport was the most critical point to become infected. The study by Benavides *et al.* (2020) concluded that introduction of BVDV and BHV-1 could be reduced by the implementation of biosecurity measures. According to Foddai *et al*. (2014) introduction of BVDV in Danish dairy herds could be reduced from 10.7 % to 2.9 % by compulsory testing of imported animals and disinfection of tools used by hoof trimmers abroad. *Mycoplasma bovis* introduction to farm clearly increases when cattle are imported, and the most important risk factor is large herd size (Nicholas *et al*., 2016). Movement of animals, because of trade or showing is important risk to clinical disease caused by *M. bovis* (Aebi *et al*., 2015).

Gates *et al*. (2013) found out that in addition to cattle movement, visitors have an important role in transmission of pathogens among farms. Larger herds have more visitors, so movement of people are associated with herd size (Nöremark *et al*., 2013). Amelung *et al*. (2018) found out that the risk of BVDV entry to the farm is related to the number of contacts of people and traffic on dairy farms. Those include inseminator, hoof trimmer, vehicles of the rendering facilities, milk collection trucks, feed suppliers, neighboring cattle farmers, and veterinarians. According to Gates *et al.* (2013) the most reported risk factors for local spread of the BVDV infection were the presence of public footpaths, shared ponds, and deer grazing in proximity. Increasing herd size is positively associated with seropositivity of BRSV (Toftaker *et al*., 2016).

Oma *et al.* (2018) argued that it is possible that humans carry BRSV virus on their nasal mucosa for short period of time. Still the importance of this indirect transmission is less important than that of contaminated fomites. The level of viral particles of BRSV recovered from boots was lower than from coats, which can be explained by rinsing the boots with water. Herds that do not provide boots for visitors have increased risk of seropositivity for BRSV so rinsing the boots with water does not sufficiently prevent virus transmission between herds (Ohlson *et al*., 2010). Lack of boots provision for visitors is found to be risk factor for BRSV infection (Beaudeau *et al*., 2010). Human carrying items like stethoscopes and wristwatches are often brought between farms without washing and disinfection. Those fomites can carry infective virus particles for at least 24 h after exposure to infected herds (Oma *et al*., 2018). Likelihood of indirect transmission of BRSV by fomites decreases with increasing travelling time and therefore distance, so the number of infective virions on equipment decreases over time (Toftaker *et al*., 2016). Herd-specific clothing and equipment are biosecurity measures for prevention of disease spread. Washing and disinfecting fomites brought between farms will decrease the risk of disease transmission (Oma *et al*., 2018). Fecteau (2017) found out that farm equipment, boots and clothes contaminated with feces could transmit paratuberculosis in theory to new herd.

1.7.2. Biocontainment of cattle pathogens

Lack of within-farm biosecurity would increase the risk of disease transmission between different management groups. This may create persistently infected adults exposing youngstock to pathogens. Farm personnel moving between different management groups without any cleaning protocols or changing protective clothes and boots will increase the risk of disease transmission within the farm. Tractors are usually used in different tasks on a farm, so those also bear potential risk for disease transmission (Brennan and Christley, 2012). According to Sarrazin *et al*. (2014) study in Belgian cattle farms the working lines from young to old cattle was generally not followed and sick animals were housed usually in a calving box without cleaning and disinfection of the box.

Nielsen *et al*. (2012) found out that barn hygiene level and herd susceptibility were influenced by the probability of spreading the *S. Dublin* infection within the herd, duration of infection, probability of extinction and epidemic size. In larger herd's hygiene management and housing practices were more important than in smaller herds even though the herd size did not affect the probability of infection spread upon exposure.

High total amount of milk produced in a farm and stress factors like moldy feed, high in-barn temperature and overcrowding are correlated with *M. bovis* infection (Aebi *et al*., 2015).

2. AIMS OF THE STUDY

Aims of this thesis were:

- To analyze the prevalence of BHV-1, BVDV, BRSV, *M. bovis*, *S.* Dublin and MAP infections in Estonian dairy cattle herds;
- To reveal the frequency of implementation of selected external biosecurity measures in large-scale Estonian dairy cattle herds;
- And to find associations between implementation of external biosecurity measures and herd status of selected endemic infections.

3. MATERIALS AND METHODS

3.1. Study design and sample collection

In total, 120 dairy cattle herds in Estonia were included in the present cross-sectional study. The inclusion criterion for the study herds were herd size of at least 100 dairy cows, freestall keeping system for lactating cows and not aiming to cease the production in near future. The list of herds meeting the herd size criterion was obtained from the Estonian Livestock Performance Recording Ltd (ELPR) in January 2019. The number of herds meeting the criterion was 182, from which the study herds were selected based on random sampling using random number generator in Stata® MP 14.2 (StataCorp, Texas). Mainly farm managers were contacted by phone and the study aims and methodology were briefly explained for them. Herds meeting the other two inclusion criterions were specified and in case of compliance and agreement the herd visit to the respective herd was performed by one of two veterinarians. Herds, which were included in the study comprised roughly 66 % of the total target population.

All the 120 study herds were visited once between August 2019 and July 2020. Face-to-face interview was conducted with a farm manager or veterinarian during the herd visit. In addition to other study subjects not covered in the present study, biosecurity practices and vaccination programs implemented within the herd during the last three years were recorded by using a questionnaire. From each study herd 10 serum samples were taken from randomly selected heifers 8-16 months of age and bulk tank milk (BTM) samples were collected from each bulk tank on the farm. In total, one bulk tank was present in 89 farms, 30 farms had two milk tanks and one farm had five milk tanks. Serum and BTM samples were refrigerated to the temperature +4 ºC and sent to the laboratory where serum was separated from the blood samples before freezing.

For each 120 study herds the data about the average number of cows in the herd at the year before farm visit and 305-days average herd-level milk yield was obtained from the Estonian Livestock Performance Recording Ltd (ELPR).

3.2. Sample analysis

Herd infection status of each study herd regarding bovine herpesvirus 1 (BHV-1), bovine viral diarrhea virus (BVDV), bovine respiratory syncytial virus (BRSV), *Mycoplasma bovis* (*M.*

bovis), *Salmonella* Dublin (*S.* Dublin) and *Mycobacterium avium* subsp. *paratuberculosis* (MAP) was assessed by using the heifers' serum samples and BTM samples determining disease antibodies using commercial ELISA tests. Table 1 shows diagnostic tests used according to the manufacturer's instructions for each investigated pathogen and cut-off values for positive test results.

Table 1. Diagnostic tests used for testing heifers' serum samples and bulk tank milk samples for the presence of disease-specific antibodies

Pathogen	Diagnostic test for antibodies	Results calculation	Cut-off for positive result*
Bovine Herpesvirus 1	IDEXX IBR gB X3 (Idexx Laboratories, Inc.)	Blocking %	≥45
	IDEXX IBR gE (Idexx Laboratories, Inc.)	S/N ratio	≤0.70 (serum) ≤0.80 (milk)
Bovine Viral Diarrhea Virus	IDEXX BVDV Total Ab, (Idexx Laboratories, Inc.)	S/P ratio	≥0.20
Bovine Respiratory Syncytial Virus	SVANOVIR BRSV-Ab, Boehringer Ingelheim, Svanova	Percent Positivity	≥10
Mycoplasma bovis	Monoscreen Ab ELISA, (Bio-X Diagnostics S.A.	Percent Positivity	≥37
Salmonella Dublin	PrioCHECK Salmonella Ab bovine Dublin (Prionics AB)	Percent Positivity	≥35
Mycobacterium avium subsp. *paratuberculosis*	*Mycobacterium paratuberculosis* Test Kit for Cattle PARACHEK 2 (Prionics AG)	Percent Positivity	≥15 (serum) ≥10 (milk)

* suspect test results were considered positive in the data analysis

3.3. Data analysis

IBR glycoprotein B (gB) and IBR glycoprotein E (gE) ELISA tests were used for testing BHV-1 antibodies in non-vaccinated and vaccinated herds, respectively. According to the questionnaire data, vaccinated herds had used BHV-1 marker vaccines in their farms within the last five years. Due to possibility of discriminating disease antibodies from those induced by marker vaccines, vaccinated herds were included into BHV-1 prevalence calculation. Herds vaccinated against BVDV and BRSV were excluded from the respective disease prevalence

calculations. All ELISA test results were dichotomized into negative or positive based on the test cut-off values. Suspect ELISA test results were considered positive in the data analysis.

ELISA test results of the heifers' serum samples and BTM samples regarding herd infection status of the six investigated pathogens were inserted to Microsoft® Excel® 365, version 2102. The herd infection status of each pathogen was dichotomized into positive or negative based on heifer serum and BTM samples' test results. The herd was considered positive if it had at least one positive heifers' serum sample or BTM sample test result for the respective disease. In case of only one suspect heifer test result and all other samples being negative, the herd was considered as negative for the respective pathogen. For three herds in which only one out of ten tested heifers gave suspect test result of BVDV and BTM samples tested BVDV negative the herd was considered BVDV negative in further analysis. The apparent herd prevalence together with binominal exact 95 % Confidence Intervals (CI) for the six infectious diseases were calculated as a sum of the heifer and cow tests by using '*cii*' command in Stata® MP 14.2 (StataCorp, Texas).

Data of herd sizes, 305-days average herd-level milk yields, barn types and milking systems, and herd biosecurity practices were entered to the Microsoft® Excel® 365, version 2102. Biosecurity measures consisted data of purchasing animals, cattle grazing or using outside walking yards, constant movement of cattle between associated farm units, pasture contacts with other herds and participation in animal shows. Human actions on the farms were examined with questions concerning veterinarian or artificial insemination (AI) technician visiting other farms, employees visiting other farms or distinct cattle units belonging to the same owner, employees changing clothing before entering the farm, support service providers disinfecting equipment, and farms providing protective clothing to visitors, availability of hand disinfection and disinfection baths/mattresses in the farm entrances. Distance of the carcass loading place from the farm was categorized into <10 meters, 10–19 meters, 20–99 meters and ≥100 meters to allow reasonable number of study farms allocated into each category. In total 13 pie charts were created of each biosecurity practice to illustrate the distribution of their implementation on the study farms.

The association between the implemented biosecurity practices and herd status regarding infectious diseases were investigated by using logistic regression analysis. Herds that did not report any vaccinations for the specific disease were included. Univariable logistic regression models ('*logit*' command) were created for each studied pathogen using herd status of the

pathogen (negative/positive) as an outcome variable and biosecurity and farm characteristics as predictor variables. The results of the univariable logistic regression analysis were used to screen variables for including into multivariable logistic regression analysis. Variable inclusion criterion for multivariable modelling was a p-value <0.2. Multivariable logistic regression models were created by removing insignificant variables from the model one-by-one, whereas variable 'herd size' was controlled in the models as a confounder. Statistically significant associations were confirmed at p-value <0.05 and associations showing tendencies were considered at the significance level 0.05≤ p-value ≤0.20.

4. RESULTS

4.1. Characteristics of the study herds

In total, 120 Estonian dairy cattle herds distributed over the country were included in the study (Figure 1). The mean herd size was 518 dairy cows (range 92–2,275) and 305-days average herd-level milk yield was 10,319 kg (range 5,983–13,155). All the herds had freestall keeping system for lactating cows and those were categorized into four different barn types. Over half (57.5 %) of the study herds had semi-insulated barn. Non-insulated open-air barns were in 27.5 % and insulated barn in 11.7 % of the herds. Different types of barns like both insulated and non-insulated barns or semi-insulated and insulated barns were in 3.3 % of the study herds. Parallel milking parlor was the most common milking system as 45.0 % of the herds had it. Robot milking system was used in 28.3 %, fishbone milking parlor in 13.3 % and carousel milking parlor in 5.8 % of the study herds. Eight herds (6.7 %) had combined milking systems in use and in one farm the cows were milked in a robot carousel milking parlor.

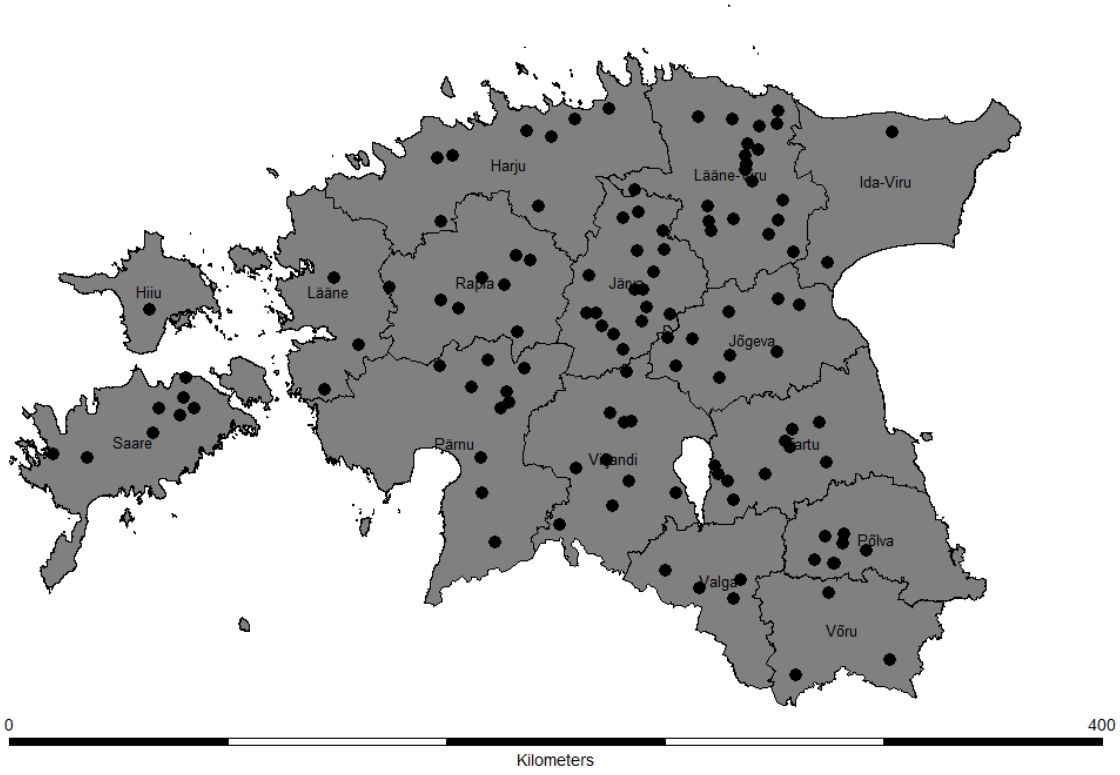

Figure 5. Locations of 120 study herds (black dots) in Estonia visited between August 2019 and July 2020.

4.2. Infectious diseases prevalence of study herds

In total, 1,200 serum samples were collected from heifers aiming to be age of 8-16 months. Three (0.25 %) of the tested heifers turned out to be between six to seven months old, 93 (7.8 %) were older than 16 months, and the age remained unknown for three sampled heifers. Study herds had one to five bulk tanks per farm and the total number of bulk tank milk samples collected was 154.

Based on heifers' serum samples and BTM samples 68 herds were BHV-1 seropositive and the apparent herd prevalence was 56.7 % (95% CI 47.3; 65.7) in all 120 study herds (Table 2, Figure 2). There were 31 (25.8 %) farms, which vaccinated their cattle against BHV-1. In total, 89 herds had not vaccinated their cattle against BHV-1 within the last five years. Among those non-vaccinated herds 63 were BHV-1 positive (70.8 %, 95 % CI 60.2; 79.9) (Table 2).

Nine out of 120 study herds stated they had vaccinated their cattle against BVDV and were excluded from the herd prevalence calculations. Altogether, 111 dairy herds were tested for BVDV, from which 30 herds gave seropositive test result resulting with apparent herd prevalence of 27.0 % (95 % CI 19.0; 36.3) (Table 2, Figure 2).

There were 25 herds out of 120, which vaccinated their cattle against BRSV and were excluded from the herd prevalence calculations. Based on heifers' serum samples and BTM samples only five herds tested seronegative resulting with BRSV apparent herd prevalence of 94.7 % (95 % CI 88.1; 98.3) (Table 2, Figure 2).

Based on heifer and cow testing in 120 herds *M. bovis* positive herds were 58 (48.3 %, 95 % CI 39.1; 57.6), *S.* Dublin positive herds 29 (24.2 %, 95 % CI 16.8; 32.8) and three herds tested MAP positive giving herd apparent prevalence for MAP 2.5 % (95 % CI 0.5; 7.1) (Table 2, Figure 2).

Table 2. Herd prevalence of selected pathogens of 120 Estonian dairy herds based on 10 heifers' serum samples of each herd and herds' BTM samples of each bulk tank on the farm

Pathogen	Number of herds tested	Number of positive herds	Apparent herd prevalence, % (binominal exact 95% CI)
BHV-1 (v+ and v-)[a]	120	68	56.7 (47.3; 65.7)
BHV-1 (v+)[b]	31	5	16.1 (5.5; 33.7)
BHV-1(v-)[c]	89	63	70.8 (60.2; 79.9)
BVDV[d]	111	30	27.0 (19.0; 36.3)
BRSV[e]	95	90	94.7 (88.1; 98.3)
M. bovis[f]	120	58	48.3 (39.1; 57.6)
S. Dublin[g]	120	29	24.2 (16.8; 32.8)
MAP[h]	120	3	2.5 (0.5; 7.1)

[a]Bovine herpesvirus 1, vaccinated (v+) and non-vaccinated (v-) herds
[b]Bovine herpesvirus 1, vaccinated (v+) herds
[c]Bovine herpesvirus 1, non-vaccinated (v-) herds
[d]Bovine viral diarrhea virus
[e]Bovine respiratory syncytial virus
[f]*Mycoplasma bovis*
[g]*Salmonella* Dublin
[h]*Mycobacterium avium* subsp. *paratuberculosis*

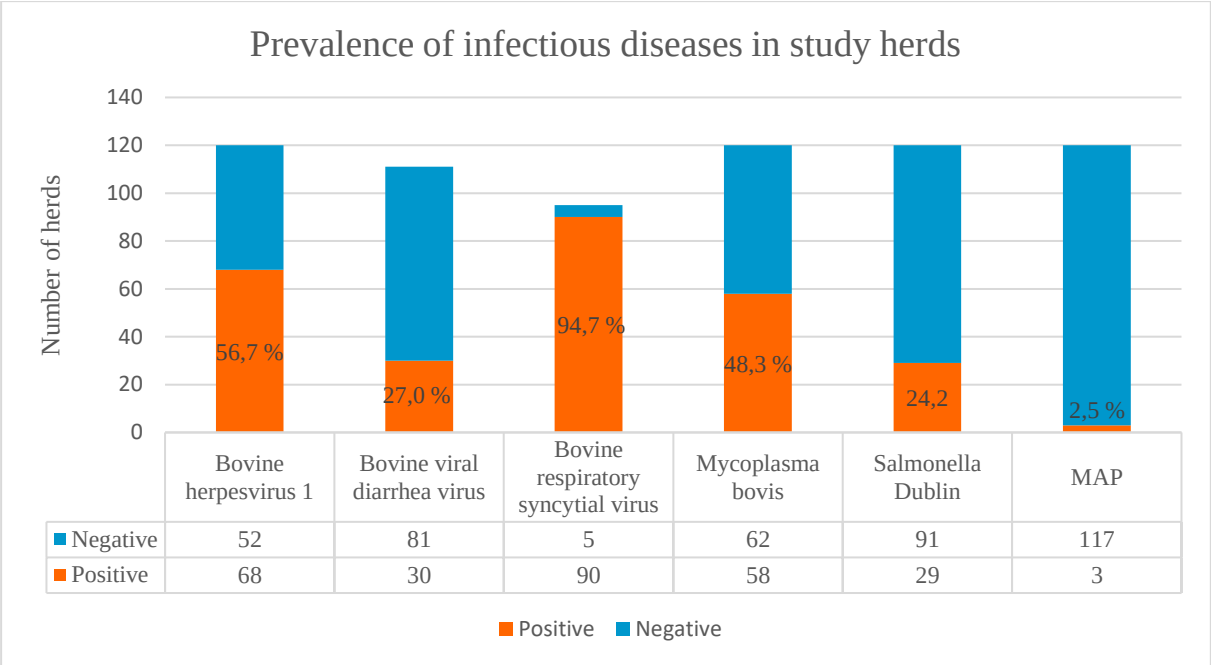

Figure 6. Illustration of each investigated pathogen prevalence (denoted as percentage on the histogram bars) on the tested study herds.

4.3. Biosecurity measures on study herds

Almost half of the 120 study farms (n= 59, 49 %) had purchased new animals over the last three years at least once and 61 farms (51 %) did not introduce new animals to the farm (Figure 7). Constant movement of cattle between associated units (e.g., between cow and youngstock barns), were reported in 26 farms (22 %) (Figure 8).

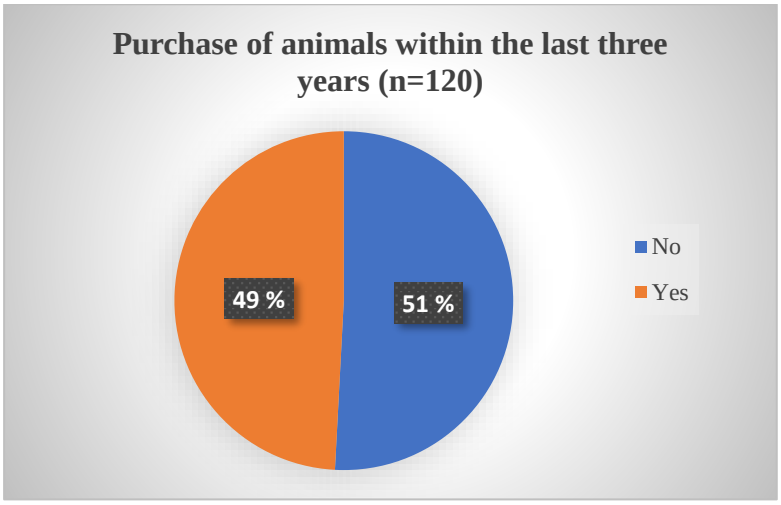

Figure 7. Distribution of study herds based on purchasing animals within the last three years

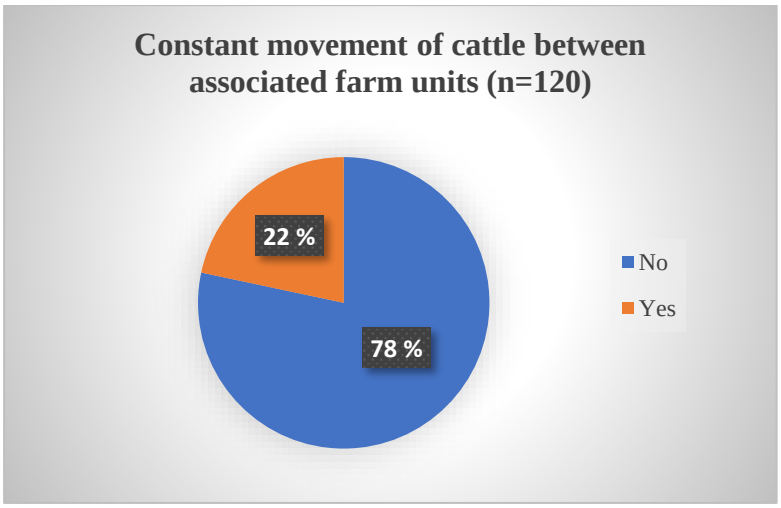

Figure 8. Distribution of study herds based on constant movement of cattle between associated farm units

In three fourth of the farms (n= 90, 75 %) cattle were grazed or allowed to go to outside walking yards (Figure 9), but pasture or yard area contact with other herds occurred only in 10 % (n= 12) of the farms (Figure 10).

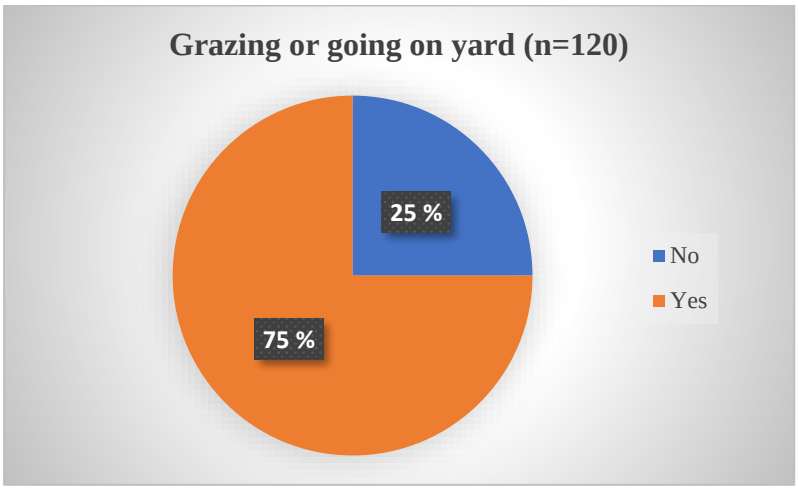

Figure 9. Distribution of study herds based on cattle grazing or going outside on yard

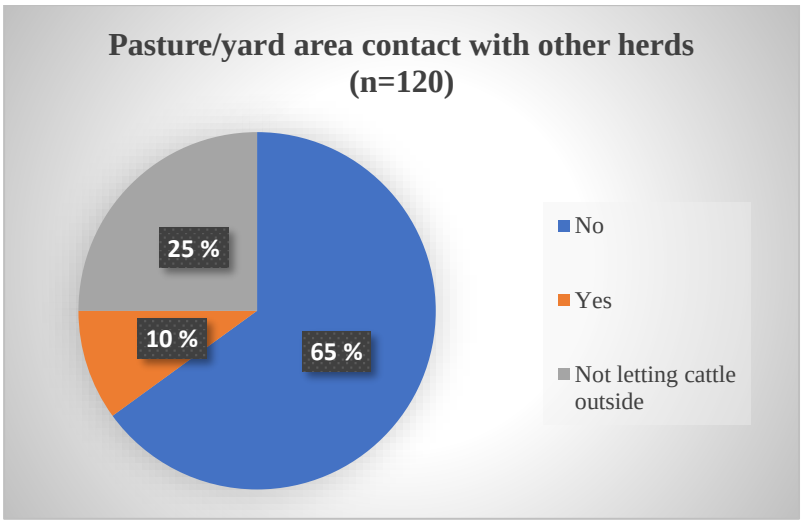

Figure 10. Distribution of study herds based on pasture/yard area contact between distinct herds

In total, 18 % (n= 22) of the farms had participated in animal shows with their cattle within the last three years and 98 (82 %) of the farms didn't have that kind of action (Figure 11).

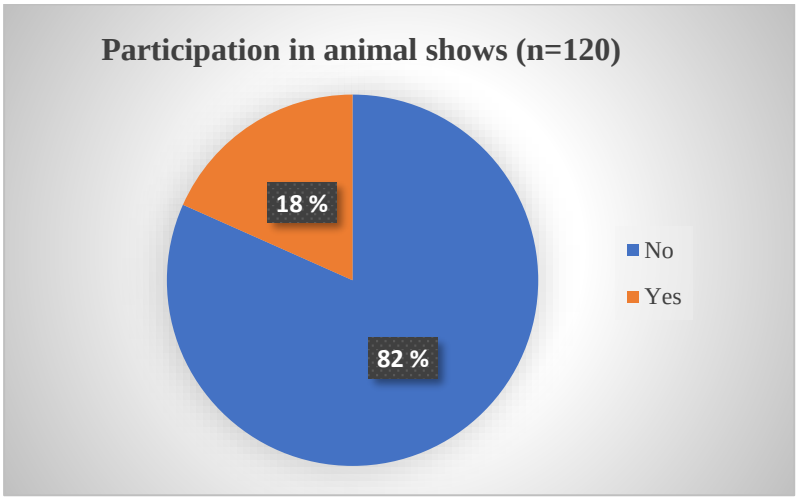

Figure 11. Distribution of study herds based on participating in animal shows with their cattle within the last three years

Employees visited other herds or distinct cattle units belonging to the same owner in 85 % (n= 102) of the study herds and veterinarian or artificial insemination (AI) technician provided service to other herds in 74 % of the study herds (n= 89) (Figure 12 and 13, respectively). In total, 92 % (n= 110) of the farms answered that employees always changed their clothes when entering the farm. In 8 % (n= 10) of the farms the employees changed clothes sometimes or never when entering the farm (Figure 14). Protective clothes and boots were always provided for visitors in 59 % (n= 71) of the herds and in 41 % (n= 49) of the herds protective clothing were not routinely provided for visitors (Figure 15). Support service providers (i.e. veterinarian,

AI-technician, hoof trimmer and consultant) disinfected their equipment before entering the farm always in 82 % (n= 98) of the farms and sometimes or never in 18 % of the farms (Figure 16).

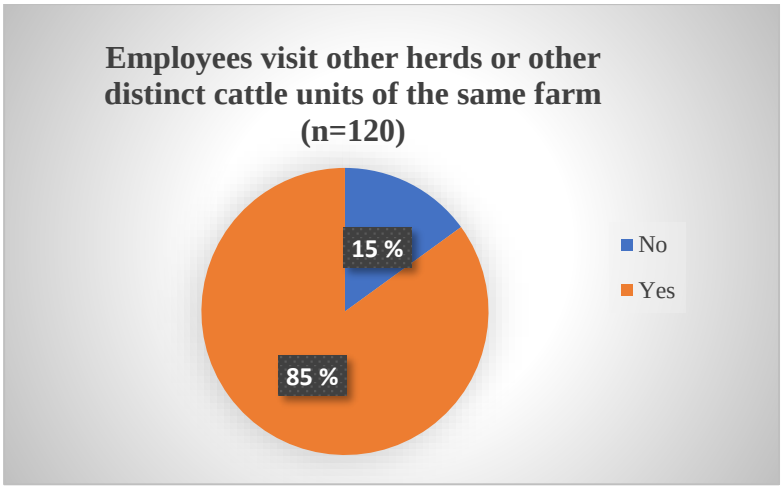

Figure 12. Distribution of study herds based on employees visiting other herds or distinct cattle units belonging to the same farm owner

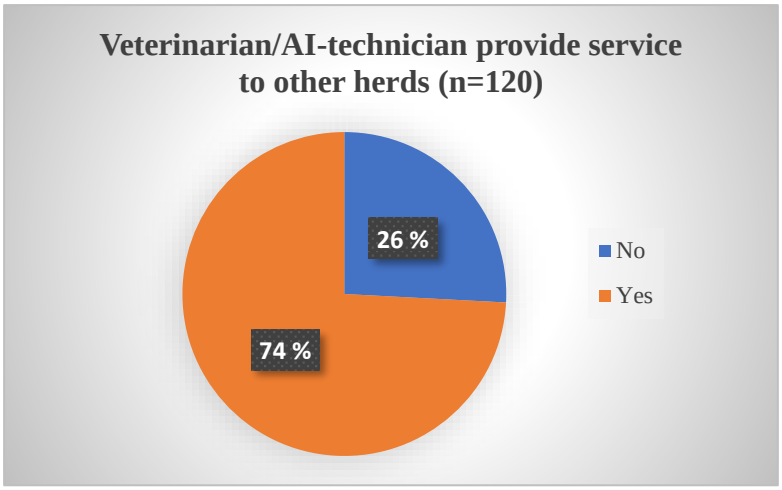

Figure 13. Distribution of study herds based on veterinarian/artificial insemination (AI) technician providing service to other herds

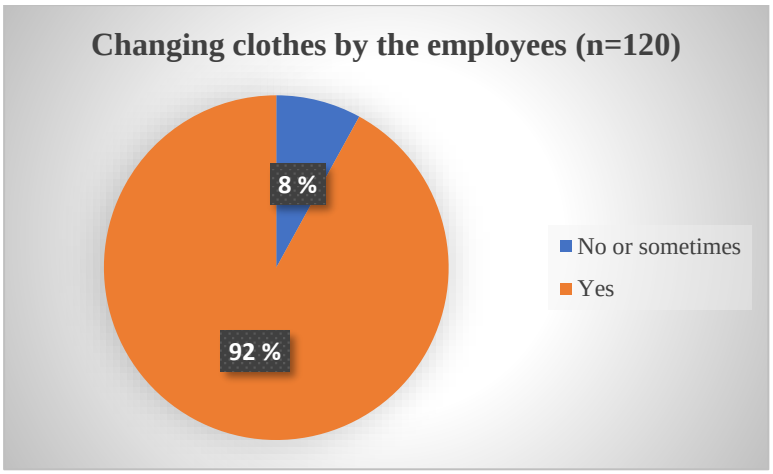

Figure 14. Distribution of study herds based on changing clothes by the employees

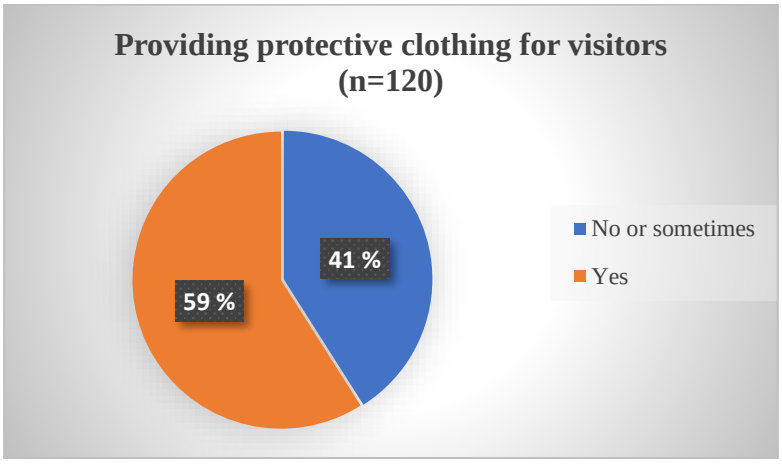

Figure 15. Distribution of study herds based on farm's providing protective clothes and boots for visitors

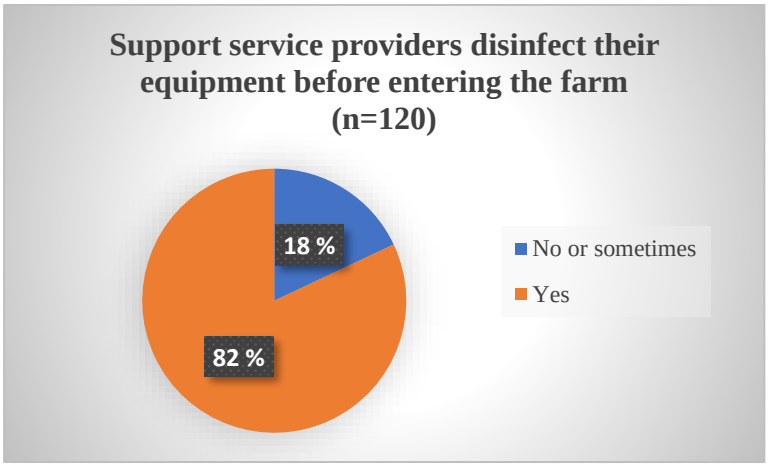

Figure 16. Distribution of study herds based on support service providers disinfecting their equipment before entering the farm

Disinfection mattresses or baths were not available at the farm entrance in 75 % (n= 90) of the farms (Figure 17), and also hand disinfection was not provided in the entrances of 87 % (n= 104) of the farms (Figure 18). Figure 19 shows the distance of carcass loading place from the farms in meters. In 35 of the farms (29 %) the carcass loading place was under 10 meters away from the farm. In 21 of the farms (18 %) the distance was 10-19 meters, in 40 farms (33 %) the distance was 20-99 meters and having distance to carcass loading place at least 100 meters or more had 24 (20 %) of the farms.

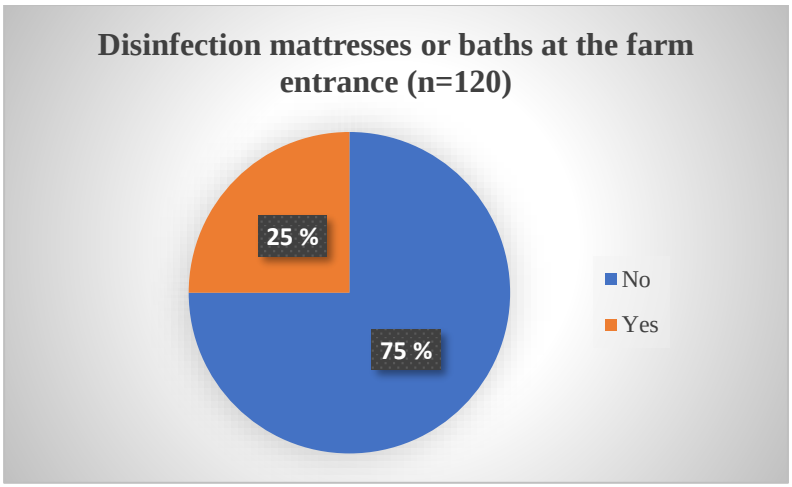

Figure 17. Distribution of study herds based providing disinfection mattresses or baths at the farm entrance

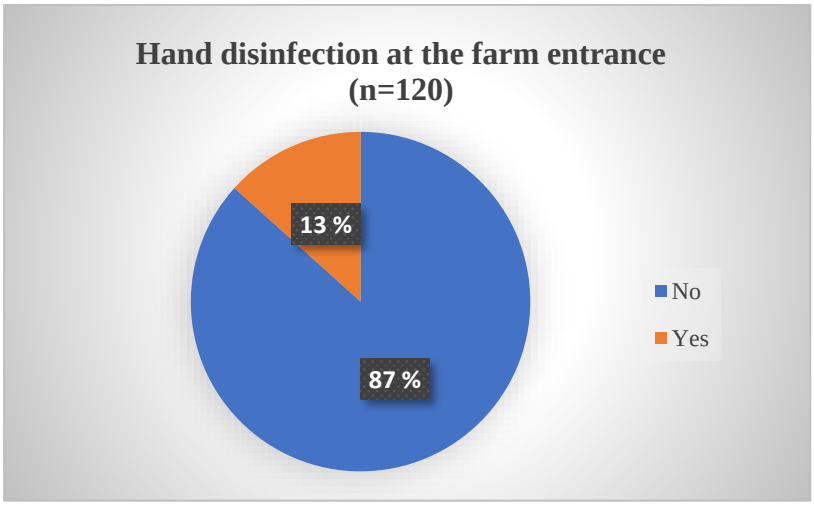

Figure 18. Distribution of study herds based on providing hand disinfection at the farm entrance

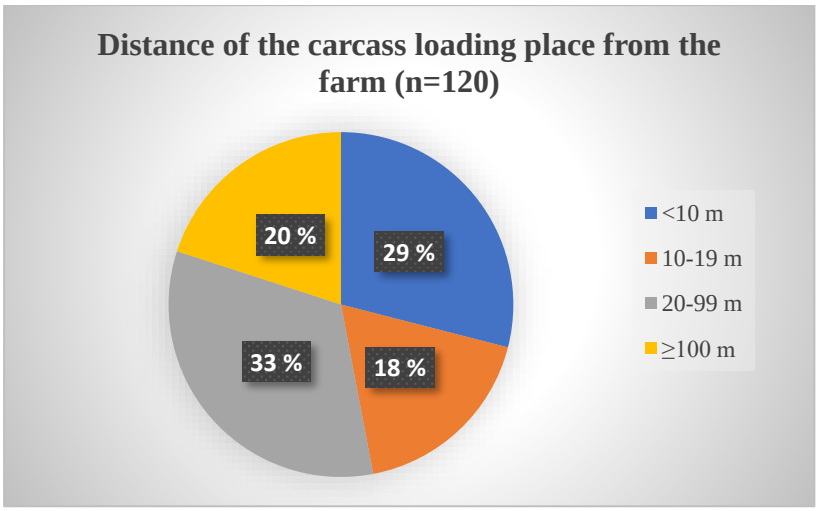

Figure 19. Distribution of study herds based on distance (meters) of the carcass loading place from the farm

4.4. Association between biosecurity-related risk factors and investigated herd infectious diseases

Due to the presence of only five BRSV negative herds among 95 tested ones and three MAP positive herds out of 120 tested herds, these diseases were not included into further statistical analyses finding associations with farm biosecurity measures.

Univariable logistic regression analyses are presented in Table 3. Biosecurity factors that were further introduced to subsequent multivariable models for each disease are shown in colors.

Table 3. Univariable logistic regression analyses results for screening biosecurity-related risk factors and farm characteristics for herd infectious diseases in non-vaccinated study herds

Biosecurity measure	Bovine herpesvirus 1	Bovine viral diarrhea virus	*Mycoplasma bovis*	*Salmonella* Dublin
Purchase of animals within the last three years	0.78 (0.31; 1.95) p=0.593	1.36 (0.59; 3.15) p=0.474	1.60 (0.78; 3.28) p=0.204	0.79 (0.34; 1.84) p=0.592
Constant movement of cattle between associated farm units	1.20 (0.38; 3.76) p=0.754	2.05 (0.78; 5.41) p=0.147	0.73 (0.31; 1.76) p=0.488	0.93 (0.33; 2.58) p=0.883
Grazing of cattle or going on yard	1.18 (0.42; 3.34) p=0.757	0.34 (0.14; 0.86) p=0.022	1.57 (0.68; 3.63) p=0.293	1.06 (0.40; 2.81) p=0.902
Pasture/yard area contacts with other herds	0.62 (0.13; 2.90) p=0.544	2.86 (0.71; 11.50) p=0.140	0.43 (0.12; 1.54) p=0.195	1.67 (0.45; 6.18) p=0.445
Participation in animal shows	3.12 (0.65; 14.94) p=0.154	1.46 (0.52; 4.05) p=0.471	2.15 (0.83; 5.58) p=0.117	2.10 (0.78; 5.66) p=0.145
Employees visiting other farms or distinct cattle units of the same farm	2.53 (0.81; 7.92) p=0.110	1.36 (0.41; 4.51) p=0.617	0.71 (0.26; 1.95) p=0.507	1.14 (0.34; 3.77) p=0.835
Veterinarian/AI-technician provide service to other herds	1.18 (0.42; 3.34) p=0.757	0.82 (0.32; 2.06) p=0.668	0.28 (0.12; 0.67) p=0.005	1.13 (0.43; 2.97) p=0.811
Changing clothes by the employees	0.67 (0.13; 3.45) p=0.629	0.52 (0.14; 1.99) p=0.339	1.45 (0.39; 5.41) p=0.583	1.30 (0.26; 6.50) p=0.748
Providing protective clothing for visitors	0.33 (0.12; 0.93) p=0.036	0.44 (0.19; 1.03) p=0.058	0.96 (0.46; 1.98) p=0.906	0.38 (0.16; 0.90) p=0.028
Support service providers disinfect their equipment before entering the farm	0.50 (0.13; 1.93) p=0.316	0.91 (0.32; 2.61) p=0.860	0.36 (0.14; 0.97) p=0.044	0.29 (0.11; 0.77) p=0.013
Disinfection mattresses or baths at the farm entrance	1.2 (0.38; 3.76) p=0.754	1.40 (0.55; 3.56) p=0.482	0.64 (0.28; 1.48) p=0.293	0.94 (0.36; 2.49) p=0.902
Hand disinfection at the farm entrance	0.53 (0.15; 1.84) p=0.313	0.98 (0.29; 3.35) p=0.973	0.31 (0.09; 1.02) p=0.054	0.69 (0.18; 2.62) p=0.588
Distance of the carcass loading place from the farm	1.00 (1.00; 1.00) p=0.327	1.00 (1.00; 1.00) p=0.807	1.00 (1.00; 1.00) p=0.265	1.00 (1.00; 1.00) p=0.983
Herd size	1.12 (1.01; 1.24) p=0.031	1.09 (1.03; 1.16) p=0.004	1.09 (1.02; 1.16) p=0.006	1.08 (1.02; 1.15) p=0.007
Herd milk yield	1.15 (0.95; 1.38) p=0.145	1.16 (0.96; 1.40) p=0.134	1.08 (0.92; 1.26) p=0.338	1.22 (0.99; 1.49) p=0.060

According to multivariable logistic regression model, herds where employees visited other farms or other distinctly operating units belonging to the same owner had on average 4.39 times higher risk to have BHV-1 infection in their herd (95 % CI 1.13; 17.09, p = 0.033). Herds, in which the visitors used protective clothing had on average 54 % lower chance to be BHV-1 positive compared to the herds where protective clothing were not always provided for visitors (OR= 0.46, 95 % CI 0.15; 1.39, p = 0.169). Herds with larger herd size were having 1.13 times higher chance to have BHV-1 infection compared to the herds, where herd size was smaller by 50 cows (95 % CI 1.01; 1.26, p = 0.035) (Table 4).

Table 4. Biosecurity-related risk factors for herd bovine herpesvirus-1 infection in 89 non-vaccinated Estonian dairy herds

Risk factor	Category	Herds (n)	OR	95 % CI	p-value
Employees visiting other farms or distinct cattle units of the same farm	No	15	1		
	Yes	74	4.39	1.13; 17.09	0.033
Providing protective clothing for visitors	No/sometimes	36	1		
	Yes	53	0.46	0.15; 1.39	0.169
Herd size (increase of the herd size by 50 cows)		120	1.13	1.01; 1.26	0.035

Letting cattle out to pastures or walking yards (OR = 0.27, 95 % CI 0.10; 0.72, p = 0.009) and providing protective clothing for visitors (OR = 0.48, 95 % CI 0.19; 1.20, p = 0.115) were protective factors for herd positive status of BVDV. There was a 1.10 times higher chance to have BVDV infection in the larger herds compared to herds with a smaller herd size (OR = 1.10, 95 % CI 1.03; 1.17, p = 0.003 by increase of 50 cows in a herd) (Table 5).

Table 5. Biosecurity-related risk factors for herd bovine viral diarrhea virus infection in 111 non-vaccinated Estonian dairy herds

Risk factor	Category	Herds (n)	OR	95 % CI	p-value
Letting the cattle on pasture or walking yards	No	27	1		
	Yes	84	0.27	0.10; 0.72	0.009
Providing protective clothing for visitors	No/sometimes	43	1		
	Yes	68	0.48	0.19; 1.20	0.115
Herd size (increase of the herd size by 50 cows)		111	1.10	1.03; 1.17	0.003

Protective factor for *M. bovis* infection in herd was found out to be veterinarian/artificial insemination (AI) technician providing service to other herds (OR = 0.28, 95 % CI 0.11; 0.73, p = 0.009). Risk to have *M. bovis* infection was lower in herds where support service providers disinfected their equipment before entering the farm (OR = 0.35, 95 % CI 0.12; 1.002, p = 0.051). Herds in which hand disinfection was available at the farm entrance had somewhat lower risk to have *M. bovis* infection present in their herds (OR = 0.42, 95 % CI 0.12; 1.48, p = 0.178), but it was not statistically significant. Having a large herd size, the chance to be infected with *M. bovis* was 1.07 times higher than in the herds with a smaller herd size (OR= 1.07, 95 % CI 1.003; 1.14, p = 0.041 by increase of 50 cows in a herd) (Table 6).

Table 6. Biosecurity-related risk factors for herd *Mycoplasma bovis* infection in 120 Estonian dairy herds

Risk factor	Category	Herds (n)	OR	95 % CI	p-value
Veterinarian/AI-technician provide service to other herds	No	31	1		
	Yes	89	0.28	0.11; 0.73	0.009
Support services disinfect their equipment before entering the farm	No/sometimes	22	1		
	Yes	98	0.35	0.12; 1.002	0.051
Hand disinfection at the farm entrance	No	104	1		
	Yes	16	0.42	0.12; 1.48	0.178
Herd size (increase of the herd size by 50 cows)		120	1.07	1.003; 1.14	0.041

Table 7 shows that herd chance to be *S.* Dublin positive was on average 2.28 times higher in those herds which had participated in animal shows with their cattle within the last three years (95 % CI 0.76; 6.78, p = 0.140).

Table 7. Biosecurity-related risk factors for herd *Salmonella* Dublin infection in 120 Estonian dairy herds

Risk factor	Category	Herds (n)	OR	95 % CI	p-value
Participation in animal shows with their cattle within the last three years	No	98	1		
	Yes	22	2.28	0.76; 6.78	0.140
Providing protective clothing for visitors	No/sometimes	49	1		
	Yes	71	0.43	0.17; 1.11	0.083
Support services disinfect their equipment before entering the farm	No/sometimes	22	1		
	Yes	98	0.36	0.12; 1.03	0.057
Herd size (increase of the herd size by 50 cows)		120	1.07	1.01; 1.13	0.027

Herds which provided protective clothing for visitors and where support service disinfected their equipment before entering the farm had somewhat lower chance to be *S.* Dublin positive (OR = 0.43, 95 % CI 0.17; 1.11, p = 0.083 and OR= 0.36 95 % CI 0.12; 1.03, p = 0.057, respectively). The herd risk to have *S.* Dublin infection was 1.07 times higher in larger herds compared to herds with a smaller herd size (95 % CI 1.01; 1.13, p = 0.027 by increase of 50 cows in a herd) (Table 7).

5. DISCUSSION

5.1. Herd prevalence of selected infectious diseases

This is the first broader study conducted in Estonia revealing the spread of several economically important cattle pathogens that deteriorate the health and welfare of cattle. One of the studied pathogen, *S.* Dublin has a zoonotic potential so it could also impair human health (Matthews *et al.*, 2015). Even though paratuberculosis is not considered as a zoonotic disease, the organism causing paratuberculosis in cattle has also been found in humans causing Crohn's disease (OIE, 2021). Revealing the herd prevalence of important cattle pathogens is a good starting point for future monitoring of these diseases.

Using of both BTM ELISA and serum ELISA for detection of viral antibodies are valid methods to investigate the herd infection status. BTM ELISA alone might not be representative of the whole herd infection status as dry or sick cows do not contribute milk to the tank and BTM testing does not confirm or detect reinfection in a recently cleared herd (Velasova *et al*., 2017). In our study, we included testing heifers aimed to be 8 to 16 months of age in addition to BTM testing to increase the reliability of the herd testing results. At this age of cattle, the level of colostral antibodies have shown to be below their detectability level (Chamorro *et al*., 2014), and seropositive test results in young animals indicate active circulation of the disease agent within the herd. According to Houe *et al*. (2006), "spot testing" of few animals older than six to eight months is used to identify herds with presence or absence of PI animals in a herd. Sample size of 10 heifers could not detect low within-herd prevalence among heifers. It is possible that the sampling scheme used in our study could not detect infected herds with low within-herd prevalence and the presented prevalence estimates of investigated diseases might be somewhat underestimated.

Study population consisting large-scale commercial dairy cattle herds with over 100 dairy cows represented Estonian dairy herds of this size well, but the study results should not be extrapolated to herds with a smaller herd size.

BHV-1 prevalence including both vaccinated and non-vaccinated herds was 56.7 %, which is comparable to that reported by Raaperi *et al*. (2010) roughly 15 years ago in the corresponding herd size categories. Approximately one quarter of the study herds vaccinated their cattle with

marker vaccines in circumstances where there is no governmental pressure neither subsidies for BHV-1 control. This shows that Estonian dairy farmers bear BHV-1 important herd health issue and the infection possibly counteracts with their potential of selling breeding heifers for export. There was a marked difference in prevalence between BHV-1 vaccinated and non-vaccinated herds (16.1 % and 70.8 %, respectively). Although we do not know how long the sampled herds had vaccinated their cattle – a high efficacy of marker vaccination in impeding the spread of the virus within herds could be confirmed. Raaperi *et al*. (2012a) also concluded in their study that precisely followed marker vaccination program is effective way to stop the virus circulation within a herd.

BVDV was detected in 27.0 % of the tested herds and nearly all the tested herds were BRSV seropositive (94.7 %). BVDV and BRSV vaccinated herds (7.5 % and 20.8 %, respectively) were not included in the prevalence calculations, which may cause underestimation of the herd prevalence of those diseases. The latest prevalence study of selected cattle diseases in Estonian dairy herds from years 2006 to 2008 revealed that out of 100 tested herds 23 were positive to BVDV and 54 tested positive to BRSV (Raaperi *et al*., 2012b). Still, in that study 41% of the herds were smaller farms with less than 100 cows which precludes the comparison of prevalence estimates of these two studies.

M. bovis was detected in almost half of the study herds with the apparent herd prevalence of 48.3 % and according to the study of Raaperi *et al.* (2012b) endemic circulation of *M. bovis* has been confirmed in dairy cows and youngstock in Estonia over a decade ago. Regarding to *M. bovis* intramammary infection, Timonen *et al*. (2020) confirmed that in infected large-scale dairy herds, *M. bovis* clinical mastitis prevalence was 3.7 % to 11.0 %.

Previous studies according to *S.* Dublin prevalence in dairy cattle herds in Estonia has not been published. In our study, *S.* Dublin was identified in 24.2 % of the study herds. The apparent prevalence can be somewhat underestimation as BTM ELISA alone has moderate sensitivity (54 %) to detect *S.* Dublin at herd-level (Veling *et al*., 2002) and serum ELISA test performs best in animals approximately between three to ten months of age (Nielsen, 2013). Veling *et al*. (2002) concluded that combinations of serology of calves four to six months old and BTM ELISA had the highest herd-level sensitivity (99 %).

Only 2.5 % of the study herds were identified MAP positive. According to the Estonian Veterinary and Food Laboratory Annual Reports, two cattle tested MAP culture positive and low proportion of seropositive animals were confirmed – 0.2-0.8 % of seropositive tests

between the years 2016 to 2019 confirming that MAP is not widespread in Estonian dairy cattle herds (Veterinaar- ja Toidulaboratoorium, 2016; 2017; 2018; 2019). Still, our testing protocol might have somewhat underestimated the herd prevalence because the long incubation period of the pathogen causes low test sensitivity in potentially subclinically infected youngstock as well as in BTM ELISA (Collins *et al*. 2005; Divers and Peek, 2008; Fecteau, 2017).

Our study revealed that Estonian large-scale commercial dairy cattle herds are endemically infected with BHV-1, BVDV, BRSV, *M. bovis* and *S.* Dublin and only few herds have MAP-positive status. Herd prevalence of each investigated pathogen were not compared with those reported by other studies due to discrepancies of the cattle populations regarding herd sizes, farm types, vaccination coverages and used sampling schemes.

5.2. Biosecurity measures on study farms

Biosecurity measures (BSM) on farms are management strategies implemented to lower risks for infectious diseases pathogen introduction to the herd (external biosecurity) and spreading within the herd (internal biosecurity). In our study, we focused on external BSM. Infectious diseases impair negatively in different extends to animal health and welfare, productivity and economic benefits. Implementation of good biosecurity on farm is considered the most essential pillar for the control of BVDV (Lindberg and Houe, 2005) and respiratory disorders including BHV-1 and BRSV (Callan and Garry, 2002).

Purchasing live animals entail potential risk to introduce infectious diseases by direct-animal contact to herd. The farmer can lower the risk by purchasing animals from herds with known disease history and applying quarantine. Approximately half (49 %) of our study farms had purchased animals during the last three years. In Belgium, the purchasing rate was similar within five-year period according to Sarrazin *et al*. (2014). Majority of the farms had tested purchased animals before entering the farm, one fourth of the farms had quarantine stable and only six percent of the farms had at least three weeks of quarantine for their purchased animals. Sahlström *et al*. (2014) reported that in Finland purchasing of animals was also common as 54 % of their study farms had purchased live animals, but there was not mentioned the time interval for purchasing. In Denmark, herds are more closed compared to Estonia as 26.7 % of the study farms had purchased animals without quarantine within the last year (Oliveira *et al*., 2017). In our questionnaire for farm manager or veterinarian, we did not ask about implementing the

quarantine for purchased animals but according to our knowledge this is mostly not done due to absence of quarantine buildings on the farms. Still, vaccination of purchased animals might occur, but this information was not questioned due to possible inaccuracy of the information for questioned persons (mainly farm managers).

In majority of our study farms (75 %) cattle were grazed or let out to walking yards. We did not specify whether the cattle introduced to outside areas were youngstock and/or lactating cows or dry cows only due to indifference from the standpoint of introducing infectious diseases. In Spain, cattle access to pasture occurred in 43.6 % of the study farms (Villaamil *et al*., 2020) and in Denmark 64.9 % of the study farms had seasonal access to pasture for cattle (Oliveira *et al*., 2017). Based on our study, in Estonia dairy cattle had rarely pasture or yard area contact with other herds as only 10 % of the study farms reported the contact. This low contact rate is probably due to scattered cattle farm density in Estonia. For comparison, cattle had possibility for contacts with other herds over fences on pasture in 70 % of the study herds in Belgium. The same study reported possibility to other animal contact on pasture over fences and manure from other farms was very often dispersed close to the proper farm (Sarrazin *et al*., 2014). Contacts with other domestic ruminants at the pasture occurred in 51.8 % of study cattle herds in North-East and North-West Spain (Villaamil *et al*., 2020) and we acknowledge that cattle contacts with wildlife could potentially occur also in Estonia.

Attending to animal shows within the last three years was not popular among our study farms (18 %) as it was not also in Denmark where cattle attended for animal shows in 10.5 % of the study farms reported by Oliveira *et al*. (2017).

Employees (veterinarian/veterinary assistant/artificial insemination technician/other farm workers) visited other herds or distinct cattle units belonging to the same owner in 85 % of our study farms referring to very frequent and possibly risky indirect-contacts in terms of between-herd transmission of infectious diseases. Luckily, majority of our study farms (92 %) reported that employees changed clothes before entering the farm which decreases the risk of infection transmission by fomites. Constant movement of cattle between associated farm units was reported in 22 % of our study farms. In our study, we did not discriminate the movement of cattle healthcare workers (veterinarian/veterinary assistant or insemination technicians) from other workers but as veterinarian or AI-technician provided service to other herds in 74 % of our study farms the human-mediated contact rate with other herds is possibly occurring mostly via the veterinary and health care staff and less through the farm permanent workers. In

Denmark, farm staff also worked rarely (13.2 %) in another herd (Oliveira *et al*., 2017). According to previous Estonian study of Raaperi *et al*. (2010) including also smaller dairy herds, the veterinarian was an employee at the farm in roughly 36 % of the study herds.

Over half (59 %) of our study farms always provided protective clothes and boots for visitors. The similar results were received in Finland where visitors used protective clothes in 51 % and boots in 68 % of the study dairy herds based on Sahlström *et al*. (2014), but in England protective clothing was required always or almost always only in 23 % of the study farms (Nöremark *et al*., 2010). Majority of the study farms in Belgium provided clothes and boots for visitors, but they were insufficiently or incorrectly used. Protective clothes were provided in 66 % of the study farms but only 13 % reported the use of those and boots were provided in 70 % of the farms but 20 % of them used it. In Belgium not all visitors used protective clothing in the same extent (Sarrazin *et al*., 2014). The same remark was made by Nöremark *et al*. (2010; 2013) as veterinarian and AI-technician was reported to have the best practice of using protective clothing compared to salesmen, repairmen and animal transporters, who were reported to have seldom use of protective clothing. Oliveira *et al*. (2017) stated that nearly 93 % of the study farms in Denmark could have visitors that contacted with the animals without protective coveralls or overcoats and boots provided by the farm. Lack of appropriate protective clothing was reported also by Nöremark and Sternberg-Lewerin (2014) as 81 % of animal transporters reported clothing to be available on none or almost none of the farms and 76 % reported boots were on none or almost none of the farms they had visited. Based on the same study, 21 % of AI-technicians reported that protective clothing was available on almost none of the farms they had visited, and boots were available in none or almost none of the farms according to 37 % of AI-technicians opinion. Availability of boots were on none or almost none of the farms based on veterinarians (44 %) response (Nöremark and Sternberg-Lewerin, 2014). In our study, questionnaire was completed by interviewing the farm manager or veterinarian who as an overall reported rather satisfying result from the Estonian study farms providing protective clothes and boots for visitors compared to other previously named countries. Still, this biosecurity action is rather easy and cost-effective method to apply on every farm and in that way markedly reduce indirect disease introduction to herd. We did not examine the use of protective clothes between different professions on the farm in which we can assume to have variability as reported by Sarrazin *et al.* (2014).

Most of our study farms (82 %) reported that support service providers (hoof trimmer/artificial insemination technician/agricultural consultant etc.) disinfected their equipment before entering

the farm. There has no previously published studies reporting the frequency of disinfection of equipment by support service providers. Damiaans *et al*. (2020) reported only of well cleaned and disinfected obstetrical equipment before each calving among dairy farms participated in the study.

Only small proportion of our study farms provided hand disinfectants (13 %) at the farm entrance and disinfection bath/mattresses for boots were present in one fourth of the study farms. Sarrazin *et al*. (2014) reported that 61 % of the study farms had disinfection footbaths, but only at nine percent of them the footbaths were in use.

In cases where cattle die or need to be euthanized at the farm, carcass is kept outside usually covered with tarpaulin until the picking up by the carcass collector. Carcass is a risk for pathogens to spread i.e., by scavengers to herd. Having direct proximity of the carcass loading place from the farm, less than 10 meters away, was in 29 % of the study farms. Oliveira *et al*. (2017) reported 42.1 % of the study farms having pick up of carcass area 'nearby' the farm, but there was not mentioned any precise distance. Having distance to carcass loading place at least 100 meters or more had only 20 % of the study farms. Applying a leak proof closed container for carcasses until collecting from the farm would prevent infectious diseases transmission to herd (Niemi *et al*., 2016).

5.3. Biosecurity-related risk factors of herd infectious diseases

Probabilities for direct and indirect contacts in herd increases with increasing herd size and many studies have concluded that disease pressure is higher in herds with larger herd size compared to smaller herds (Bishop *et al*., 2010; Nöremark and Lewerin, 2010; Raaperi *et al.*, 2010; Amelung *et al*., 2018). Our study showed increasing risk for BHV-1, BVDV, *S.* Dublin and *M. bovis* infections when the herd size increased. Herd size was confounding variable for biosecurity related risk factors in our study. It is possible that larger herds are more likely to apply different biosecurity measures on their farms compared to smaller herds. Also, herd size itself is a proxy for many factors that were not measured in the present study.

BHV-1, BVDV and *S.* Dublin are excreted by infected animals body secretions and the pathogens can easily be transmitted by iatrogenic contact with contaminated fomites like clothes or boots (MacLachlan and Dubovi, 2017; Holschbach and Peek, 2018). In our study, BHV-1 transmission to herd was highly associated with employees visiting other farms or other distinctly operating cattle units belonging to the same owner. The risk to get BHV-1 in those

herds is probably caused by employees having close contacts with the infected animals and without sufficient hygienic procedures and changing clothes and boots between herds the virus is transmitted. Our study herds which always provided protective clothing for visitors had roughly twice as low chance to be infected by the previously named three pathogens compared to herds not always providing protective clothing. According to Ohlson *et al*. (2010), BHV-1 and BVDV transmission to herd via people or fomites can be prevented by changing clothes and boots when entering the herd. Providing protective clothes and boots for visitors was one of the biosecurity measures having the highest impact on the probability for BHV-1 or BVDV infection introduction to herd based on Benavides *et al*. (2021). Stevens *et al*. (2011) have demonstrated in experimental conditions the survival of BVDV in rubber (boots). The study revealed 88.6 % chance to recover BVDV at one hour incubation in mucus covered rubber and 46.3 % chance in phosphate buffered saline (PBS) covered rubber post-incubation. Less than 4 % chance of BVDV survival from either mucus or PBS was discovered in 48 hours post-incubation. Even though Stevens *et al*. (2011) did not discover BVDV in experimental conditions from the denim or cotton t-shirt after incubation, our study shows a potential risk to transmit the pathogen on clothes between the dairy herds in Estonia.

Grazing or letting out the cattle on walking yards might predispose them to direct contacts with neighboring herds and wildlife. Also, Hou *et al*. (2020) stated that even though there has not previous studies well documented of airborne route of BVDV infection transmission, it is possible under experimental conditions. Interestingly, herds that let their cattle out had roughly 73 % lower chance to be BVDV positive in our study. Still, looking at the univariable associations, herds that reported to have pasture/yard area contacts with other herds possible had on average 2.9 times higher risk to be BVDV positive compared to those herds in which outside reared cattle had no contacts with cattle from other herds. This means that grazing itself might not entail a high risk regarding infectious diseases but contacts with other wild or domestic ungulates might bear the risk of contracting the infection. Still, previous studies concerning BVDV transmission between cattle and wildlife does not highlight markedly the risk. Passler *et al.* (2016) reported that transmission of the infection between white-tailed deer and cattle is more likely via indirect contact for example through water ponds than directly between animals. Also, insects could act as an indirect route of BVDV transmission, but no epidemiological data are available to estimate the risk of arthropods as transmitters of BVDV infection between deer and cattle. According to Grant *et al*. (2015) rabbit poses a small but non-zero risk of BVDV infection for cattle.

Disinfection appeared to be protective against *M. bovis* infection among our study herds. In herds where support service providers disinfected their equipment (i.e., nose tongs, obstetrical-, dehorning-, and hoof paring equipment) before entering the farm had on average 65 % lower risk for *M. bovis* infection and hand disinfection at farm entrance lowered the risk to have *M. bovis* infection by 58 % compared to herds without hand disinfection possibility. Based on study by Boddie *et al*. (2002), *M. bovis* is highly susceptible to commonly used chlorine-, chlorhexidine-, acid-, and iodine-based disinfectants. In our study, disinfection of equipment was also associated with lower risk of a herd to be *S.* Dublin positive as in herds where support service providers disinfected their equipment before entering the farm had on average 64 % lower chance to be *S.* Dublin positive compared to herds where disinfection was not done. *S.* Dublin is excreted to feces, respiratory secretions and milk of infected animals (Holschbach and Peek, 2018) and without proper cleaning and disinfection of equipment those can act as mechanical vectors between herds.

Herds where veterinarian or AI-technician provided service to other herds had on average 72 % lower chance to have *M. bovis* infection compared to herds where these professions worked only in one specific herd. This indicates that cattle healthcare workers are aware of the biosecurity measures and are not possibly important fomites of cattle pathogens.

The herd risk for *S.* Dublin infection was more than doubled in our study herds if the herd had participated in animal shows within the last three years. The most common source of *S.* Dublin infection to cattle is pathogen-contaminated manure (Holschbach and Peek, 2018), so we can assume that during animal shows contaminated manure of infected cattle can be the source of infection either directly to animals or indirectly via animal handlers. Davison *et al.* (2003) reported also that participation in animal shows is a risk factor for *S.* Dublin infection spreading.

Interestingly, purchase of cattle was not a significant risk factor for any of the studied cattle pathogens. For accuracy, we limited the time-period for collecting purchase information to the last three years. This however might be too short time-period to capture the association between this measure and herd infection status. We can assume that due to large herd size and effective disease transmission environment, the identified pathogens might have been introduced to the herds even before that time. Therefore, it is not properly concluded to state that purchasing cattle is not a risk factor for the studied cattle pathogens.

Also, distance of the carcass loading place was not associated with the herd risk for the studied pathogens. Next to the distance, also the carcass handling and storage conditions as well as

cross-contamination (crossing pathways of carcass movement/storage and movement of farm vehicles and staff) might be important. Future studies should consider these aspects to analyze the effects of carcass handling to herd presence of infectious diseases and provide sound recommendations to the farmers.

5.4. Study limitations

The present study had several limitations which should be considered when interpreting the results. At first, the cross-sectional study collecting the biosecurity and infectious disease data at the same time lacks the power of making causal inferences. The causality of the identified associations should be validated in the future studies using more appropriate study design. Also, the list of measured biosecurity practices was far from complete and exhaustive list of what could be important in determining the herd risk to contract the tested infections. There are more comprehensive checklists and scoring tools available that could be used to get broader look into farm biosecurity measures and their associations with herd infections.

We also acknowledge that the used herd testing protocol might have missed the herds that had low within-herd prevalence of the infection. Individual testing of higher number of animals is needed to increase the sensitivity of the herd test.

6. CONCLUSION

Estonian large-scale commercial dairy cattle herds are endemically infected with BHV-1, BVDV, BRSV, *M. bovis* and *S.* Dublin. Farm biosecurity measures have an important role to prevent infectious disease introduction to herd. Each farm should carry out the minimum biosecurity measures by providing protective clothes and boots for employees, visitors and other staff arriving the farm and give them possibility to wash and disinfect hands on the farm. In addition, support service providers should take care of disinfection of their equipment between herds. Cattle contact with neighboring herds on pasture or yard area is preferred to diminish.

REFERENCES

Ackermann, M., Engels, M. Pro and con-tra IBR-eradication. Veterinary Microbiology, 2006, 113, 293-302.

Aebi, M., van den Borne, B.H., Raemy, A., Steiner, A., Pilo, P., Bodmer, M. *Mycoplasma bovis* infections in Swiss dairy cattle: a clinical investigation. Acta Veterinaria Scandinavica, 2015, 57, 10.

Amelung, S., Hartmann, M., Haas, L., Kreienbrock, L. Factors associated with the bovine viral diarrhoea (BVD) status in cattle herds in Northwest Germany. Veterinary Microbiology, 2018, 216, 212-217.

Beaudeau, F., Ohlson, A., Emanuelson, U. Associations between bovine coronavirus and bovine respiratory syncytial virus infections and animal performance in Swedish dairy herds. Journal of Dairy Science, 2010, 94, 1523-1533.

Beer, M., Dastjerdi, A. Manual for diagnostic tests and vaccines for terrestrial animals 2019: Infectious bovine rhinotracheitis/pustular vulvovaginitis. [Online publication] https://www.oie.int/standard-setting/terrestrial-manual/access-online/

Benavides, B., Casal, J., Diéguez, J.F., Yus, E., Moya, S.J., Armengol, R., Allepuz, A. Development of quantitative risk assessment of bovine viral diarrhea virus and bovine herpesvirus-1 introduction in dairy cattle herds to improve biosecurity. Journal of Dairy Science, 2020, 103, 6454-6472.

Benavides, B., Casal, J., Diéguez, J., Yus, E., Moya, S. J., Allepuz, A. Quantitative risk assessment of introduction of BVDV and BoHV-1 through indirect contacts based on implemented biosecurity measures in dairy farms of Spain. Preventive Veterinary Medicine, 2021, 188, 105263.

Bishop, H., Erkelens, J., Van Winden, S. Indications of a relationship between buying-in policy and infectious diseases on dairy farms in Wales. Veterinary Record, 2010, 167, 644-647.

Biswas, S., Bandyopadhyay, S., Dimri, U., H. Patra, P. Bovine herpesvirus-1 (BHV-1) – a re-emerging concern in livestock: a revisit to its biology, epidemiology, diagnosis, and prophylaxis. Veterinary Quarterly, 2013, 33, 68–81.

Boddie, R.L., Owens, W.E., Ray, C.H., Nickerson, S.C., Boddie, N.T. Germicidal activities of representatives of five different teat dip classes against three bovine mycoplasma species using a modified excised teat model. Journal of Dairy Science, 2002, 85, 1909–12.

Brennan, M. L., Christley, R. M. Biosecurity on Cattle Farms: A Study in North-West England. Plos One, 2012.

Brodersen, B.W. Bovine respiratory syncytial virus. Veterinary Clinics of North America: Food Animal Practice, 2010, 26, 323-333.

Callan, R.J., Garry, F.B. Biosecurity and bovine respiratory disease. Clinics of North America: Food Animal Practice, 2002, 18, 57–77.

Chamorro, M.F., Walz, P.H., Haines, D.M., Passler, T., Earleywine, T., Palomares, R.A., Riddell, K.P., Galik, P., Zhang, Y., Daniel Givens, M.. Comparison of levels and duration of detection of antibodies to bovine viral diarrhea virus 1, bovine viral diarrhea virus 2, bovine respiratory syncytial virus, bovine herpesvirus 1, and bovine parainfluenza virus 3 in calves fed maternal colostrum or a colostrum-replacement product. Canadian Journal of Veterinary Research, 2014, 78, 81–88.

Collins, M.T., Wells, S.J., Petrini, K.R., Collins, J.E., Schultz, R.D., Whitlock, R.H. Evaluation of five antibody detection tests for diagnosis of bovine tuberculosis. Clinical and Diagnostic Laboratory Immunology, 2005, 12, 685-692.

Damiaans, B., Renault, V., Sarrazin, S., Berge, A.C., Pardon, B., Saegerman, C., Dewult, J. A risk-based scoring system to quantify biosecurity in cattle production. Preventive Veterinary Medicine, 2020, 179, 104992.

Dargatz, D.A., Garry F.B., Traub-Dargatz, J.L. An introduction to biosecurity of cattle operations. The Veterinary Clinics Food Animal Practice, 2002, 18, 1-5.

Davison, H. C., Smith, R. P., Sayers, A. R., & Evans, S. J. Dairy farm characteristics, including biosecurity, obtained during a cohort study in England and Wales. Cattle Practice, 2003, 11, 299-310.

Divers, T.J., Peek, S.F. Diseases of dairy cattle. 2nd ed. St Louis: Elsevier Inc., 2008, pp. 99-100-102, 105-108, 250, 279-283, 363-364.

Dudek, K., Nicholas R.A.J., Szacawa, E., Bednarek, D. Mycoplasma bovis infections – occurrence, diagnosis and control. Pathogens, 2020, 9, 640.

Ellis, J.A. How efficacious are vaccines against bovine respiratory syncytial virus in cattle? Veterinary Microbiology, 2017, 206, 59-68.

Fecteau, M.E. Paratuberculosis in Cattle. Veterinary Clinics of North America: Food Animal Practice, 2017, 34, 209-222.

Foddai, A., Boklund, A., Stockmarr, A., Krogh, K., Enoe, C. Quantitative assessment of the risk of introduction of bovine viral diarrhea virus in Danish dairy herd. Preventive Veterinary Medicine, 2014, 116, 75-88.

Fulton, R., d'Offay, J., Eberle, R. Bovine herpesvirus 1: Comparison and differentiation of vaccine and field strains based on genomic sequence variation. Vaccine, 2013, 31, 1419–1471.

Gates, M.C., Woolhouse, M.E., Gunn, G.J., Humphry, R.W. Relative associations of cattle movements, local spread, and biosecurity with bovine viral diarrhoea virus (BVDV) seropositivity in beef and dairy herds. Preventive Veterinary Medicine, 2013, 112, 285–295.

Grant, D. M., Dagleish, M. P., Bachofen, C., Boag, B., Deane, D., Percival, A., Zadoks, R.N., Russell, G. C. Assessment of the rabbit as a wildlife reservoir of bovine viral diarrhea virus: serological analysis and generation of trans-placentally infected offspring. Frontiers in microbiology, 2015, 6, 1000.

Grissett, G.P., White, B.J., Larson, R.L. Structured literature review of responses of cattle to viral and bacterial pathogens causing bovine respiratory disease complex. Journal of Veterinary Internal Medicine, 2015, 29, 770-780.

Haapala, V., Pohjanvirta, T., Vähänikkilä, N., Halkilahti, J., Simonen, H., Pelkonen, S., Soveri, T., Simojoki, H., Autio, T. Semen as a source of Mycoplasma bovis mastitis in dairy herds. Veterinary Microbiology, 2018, 216, 60-66.

Holschbach, C.L., Peek, S.L. *Salmonella* in Dairy Cattle. Veterinary Clinics of North America: Food Animal Practice, 2018, 34, 133-154.

Hou, P., Xu, Y., Wang, H., He, H. Detection of bovine viral diarrhea virus genotype 1 in aerosol by a real time RT-PCR assay. BMC veterinary research, 2020, 16, 1-9.

Houe, H., Lindberg, A., Moennig, V. Test strategies in bovine viral diarrhea virus control and eradication campaigns in Europe. Journal of Veterinary Diagnostic Investigation: SAGA Journals, 2006, 18, 427-436.

Lanyon, S.R., Hill, F.I., Reichel, M.P., Brownlie J. Bovine viral diarrhoea: Pathogenesis and diagnosis. The Veterinary Journal, 2014, 199, 201-209.

Lindberg, A., Houe, H. Characteristics in the epidemiology of bovine viral diarrhea virus (BVDV) of relevance to control. Preventive Veterinary Medicine, 2005, 72, 55–73.

MacLachlan, N.J., Dubovi, E.J. Fenner's Veterinary Virology. 5th ed. Oxford: Elsevier Inc., 2017, pp. 190-195, 199-200, 353-354, 539-542.

Maunsell, F.P., Donovan, G.A. *Mycoplasma bovis* Infections in Young Calves. Veterinary Clinics: Food Animal Practice, 2009, 25, 139-177.

Matthews, T. D., Schmieder, R., Silva, G. G., Busch, J., Cassman, N., Dutilh, B. E., Green, D., Matlock, B., Heffernan, B., Olsen, G.J., Hanna, L.F., Schifferli, D.M., Maloy, S., Dinsdale, E.a., Edwards, R.A. Genomic comparison of the closely-related Salmonella enterica serovars Enteritidis, Dublin and Gallinarum. PloS one, 2015, 10.

Muylkens, B., Thiry, J., Kirten, P., Schynts, F., Thiry, E. Bovine herpesvirus 1 infection and infectious bovine rhinotracheitis. Veterinary Research, 2007, 38, 181–209.

Newcomer, B.W., Chamorro, M.F., Walz, P.H. Vaccination of cattle against bovine viral diarrhea virus. Veterinary Microbiology, 2017, 206, 78-83.

Newcomer, B.W., Givens, D. Diagnosis and control of viral diseases of reproductive importance: Infectious Bovine Rhinotracheitis and Bovine Viral Diarrhea. Veterinary Clinics of North America: Food Animal Practice, 2016, 32, 425-441.

Nicholas, R.A.J., Fox, L.K., Lysnyansky, I. Mycoplasma mastitis in cattle: To cull or not to cull. The Veterinary Journal, 2016, 216, 142-147.

Nielsen, L.R. Review of pathogenesis and diagnostic methods of immediate relevance for epidemiology and control of *Salmonella* Dublin in cattle. Veterinary Microbiology, 2013, 162, 1-9.

Nielsen, L.R., Kudahl, A.B., Østergaard, S. Age-structured dynamic, stochastic and mechanistic simulation model of *Salmonella* Dublin infection within dairy herd. Preventive Veterinary Medicine, 2012, 105, 59-74.

Nielsen, L.R., Schukken, Y.H., Gröhn, Y.T., Ersbøll, A.K. *Salmonella* Dublin infection in dairy cattle: risk factors for becoming a carrier. Preventive Veterinary Medicine, 2004, 65, 47–62.

Niemi, J. K., Sahlström, L., Kyyrö, J., Lyytikäinen, T., Sinisalo, A. Farm characteristics and perceptions regarding costs contribute to the adoption of biosecurity in Finnish pig and cattle farms. Review of Agricultural, Food and Environmental Studies, 2016, 97, 215-223.

Nöremark, M., Frössling, J., Lewerin, S. S. Application of Routines that Contribute to On-farm Biosecurity as Reported by Swedish Livestock Farmers. Transboundary and Emerging Diseases, 2010, 57, 225-36.

Nöremark, M., Frössling, J., Lewerin, S.S. A survey of visitors on Swedish livestock farms with reference to the spread of animal diseases. BMC Veterinary Research, 2013, 9, 184.

Nöremark, M., Sternberg-Lewerin, S. On-farm biosecurity as perceived by professionals visiting Swedish farms. Acta Veterinaria Scandinavica, 2014, 56, 1-11.

Ohlson, A., Emanuelson, U., Tråvén, M., Alenius, S. The relationship between antibody status to bovine corona virus and bovine respiratory syncytial virus and disease incidence, reproduction and herd characteristics in dairy herds. Acta Veterinaria Scandinavica, 2010, 52, 37.

Ohlson, A., Heuer, C., Lockhart, C., Tråvén, M., Emanuelson, U., Alenius, S. Risk factors for seropositivity to bovine coronavirus and bovine respiratory syncytial virus in dairy herds. Veterinary Record, 2010, 167, 201-6.

OIE – WAHIS Interface. Disease distribution maps. Bovine viral diarrhoea. 2019, https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap?disease_type_hidden=&disease_id_hidden=&selected_disease_name_hidden=&disease_type=0&disease_id_terrestrial=189&species_t=0&disease_id_aquatic=999&species_a=0&sta_method=semesterly&selected_start_year=2019&selected_report_period=2&selected_start_month=1&date_submit=OK, Accessed 10.10.2020.

OIE – WAHIS Interface. Disease distribution maps. Inf. Bov. rhinotracheit. (IBR/IPV). 2019, https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap?disease_type_hidden=&disease_id_hidden=&selected_disease_name_hidden=&disease_type=0&disease_id_terrestrial=37&species_t=0&disease_id_aquatic=999&species_a=0&sta_method=semesterly&selected_start_year=2019&selected_report_period=2&selected_start_month=1&date_submit=OK, Accessed 02.10.2020.

OIE – WAHIS Interface. Disease distribution maps. Paratuberculosis. 2019, https://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/Diseasedistributionmap?disease_type_hidden=&disease_id_hidden=&selected_disease_name_hidden=&disease_type=0&disease_id_terrestrial=24&species_t=0&disease_id_aquatic=999&species_a=0&sta_method=semesterly&selected_start_year=2019&selected_report_period=2&selected_start_month=1&date_submit=OK, Accessed 10.10.2020.

OIE – WAHIS Interface. Paratuberculosis, https://www.oie.int/en/disease/paratuberculosis/, Accessed 02.05.2021.

Oliveira, V. H., Sørensen, J. T., Thomsen, P. T. Associations between biosecurity practices and bovine digital dermatitis in Danish dairy herds. Journal of dairy science, 2017, 100(10), 8398-8408.

Oma, V.S., Klem, T., Tråvén, M., Alenius, S., Gjerset, B., Myrmel, M., Stokstad, M. Temporary carriage of bovine coronavirus and bovine respiratory syncytial virus by fomites and human nasal mucosa after exposure to infected calves. BMC Veterinary Research, 2018, 14, 22.

Passler, T., Ditchkoff, S. S., Walz, P. H. Bovine viral diarrhea virus (BVDV) in white-tailed deer (Odocoileus virginianus). Frontiers in microbiology, 2016, 7, 945.

Peek, S.F., Divers, T.J. Rebhun's Diseases of dairy cattle. 3rd ed. St Louis: Elsevier Inc., 2018, pp. 7.

Petersen, M.B., Pedersen, J., Holm, D.L., Denwood, M., Nielsen, L.R. A longitudinal observational study of the dynamics of *Mycoplasma bovis* antibodies in naturally exposed and diseased dairy cows. Journal of Dairy Science, 2018, 101, 7383-7396.

Raaperi, K., Nurmoja, I., Orro, T., Viltrop, A. Seroepidemiology of bovine herpesvirus 1 (BHV1) infection among Estonian dairy herds and risk factors for the spread within herds. Preventive Veterinary Medicine, 2010, 96, 74-81.

Raaperi, K., Aleksejev, A., Orro, T., & Viltrop, A. Dynamics of bovine herpesvirus type 1 infection in Estonian dairy herds with and without a control programme. Veterinary Record, 2012a, 171, 99.

Raaperi, K., Bougeard, S., Aleksejev, A., Orro, T., Viltrop, A. Association of herd BHV-1 seroprevalence with respiratory disease in youngstock in Estonian dairy cattle. Research in Veterinary Science, 2012b, 93, 641–648.

Reber, A., Reist, M., Schwermer, H. Cost-effectiveness of bulk-tank milk testing for surveys to demonstrate freedom from infectious bovine rhino-tracheitis and bovine enzootic leucosis in Switzerland. Wissenschaft Science, 2012, 154, 189-197.

Sahlström, L., Virtanen, T., Kyyrö, J., & Lyytikäinen, T. Biosecurity on Finnish cattle, pig and sheep farms – results from a questionnaire. Preventive Veterinary Medicine, 2014, 117, 59–67.

Sarrazin, S., Cay, A.B., Laureyns, J., Dewulf, J. A survey on biosecurity and management practices in selected Belgian cattle farms. Preventive Veterinary Medicine, 2014, 117, 129-139.

Stevens, E. T., Thomson, D. U., Wileman, B. W., O'Dell, S., Chase, C. C. L. The Survival of bovine viral diarrhea virus on materials associated with livestock production. The Bovine Practitioner, 2011, 45(2), 118-123.

Studdert, M.J. Bovine Herpesviruses. In: Mahy Brian, W.J., Regenmortel, Marc H.V.V., editors. Desk Encyclopedia of Animal and Bacterial Virology, 1st ed. San Diego: Elsevier Ltd., 2010, pp. 68.

Timonen, A. A., Autio, T., Pohjanvirta, T., Häkkinen, L., Katholm, J., Petersen, A., Mõtus, K., Kalmus, P. Dynamics of the within-herd prevalence of Mycoplasma bovis intramammary infection in endemically infected dairy herds. Veterinary microbiology, 2020, 242, 108608.

Toftaker, I., Sanchez, J., Stokstad, M., Nodtvedt, A. Bovine respiratory syncytial virus and bovine coronavirus antibodies in bulk tank milk – risk factors and spatial analysis. Preventive Veterinary Medicine, 2016, 133, 73-83.

Toftaker, I., Ågren, E., Stokstad, M., Nodtvedt, A., Frössling, J. Herd level estimation of probability of disease freedom applied on the Norwegian control program for bovine respiratory syncytial virus and bovine coronavirus. Preventive Veterinary Medicine, 2020, 181, 104-494.

Velasova, M., Damaso, A., Prakashbabu, B.C., Gibbons, J., Wheelhouse, N., Longbottom, D., Van Winden, S., Green, M., Guitian, J. Herd-level prevalence of selected endemic infectious diseases of dairy cows in Great Britain. Journal of Dairy Science, 2017, 100, 9215–9233.

Veling, J., Barkema, H. W., Van der Schans, J., Van Zijderveld, F., & Verhoeff, J. Herd-level diagnosis for Salmonella enterica subsp. enterica serovar Dublin infection in bovine dairy herds. Preventive veterinary medicine, 2002, 53, 31-42.

Veterinaar- ja Toidulaboratoorium. Aastaaruanded, https://www.vetlab.ee/et/loomatervis/aruanded, Accessed 30.04.2021.

Villaamil, F. J., Arnaiz, I., Allepuz, A., Molins, M., Lazaro, M., Benavides, B., Moya, S.J., Fabrega, J.C., Yus, E., Dieguez, F. J. A survey of biosecurity measures and serological status for bovine viral diarrhoea virus and bovine herpesvirus 1 on dairy cattle farms in north-west and north-east Spain. Veterinary record open, 2020, 7.

Viltrop, A., Alaots, J., Pärn, M., Must, K. Natural changes in the spread of bovine viral diarrhoea virus (BVDV) among Estonian cattle. Journal of Veterinary Medicine, Series B, 2002, 49, 263-269.

Wedderkopp, A., Strøger, U., Bitsch, V., Lind, P. Testing of bulk tank milk for Salmonella Dublin infection in Danish dairy herds. Canadian Journal of Veterinary Research, 2000, 65, 15.

Wernike K., Gethmann J., Schirrmeier H., Schröder R., Conraths F.J., Beer M. Six years (2011–2016) of mandatory nationwide bovine viral diarrhea control in Germany – a success story. Pathogens, 2017, 6, 50.

Woolums, A.R., Berghaus, R.D., Berghaus, L.J., Ellis, R.W., Pence, M.E., Saliki, J.T., Hurley, K.A., Galland, K.L., Burdett, W.W., Nordstrom, S.T., Hurley, D.J. Effect of calf age and administration route of initial multivalent modified-live virus vaccine on humoral and cell-mediated immune responses following subsequent administration of a booster vaccination at weaning in beef calves. American Journal of Veterinary Research, 2013, 74, 343-354.

APPENDIXES

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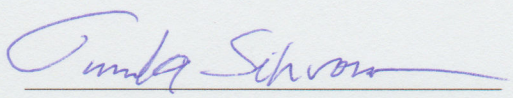
Author of the final thesis 

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